UTILISATION OF NUTRIENT RESERVES IN THE VEGETATIVE PROPAGATION OF THE GRAPEVINE

BY

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SECTION 1

INTRODUCTION

Grafting of the grapevine is a process uniting the epibiont and the hypobiont. Approximately 80 percent of the vines propagated annually in South Africa are produced by bench-grafting. According to this method, a scion with one or two buds is grafted onto the rootstock cutting. The contacting surfaces must be freshly cut without any desiccation, and the regions most likely to commence meristematic activity of both components should be in close proximity to each other. Undifferen= tiated parenchyma tissue which develops from these regions in the scion and the rootstock cutting comes into contact and, in due course, a cambial layer which connects those of the scion and rootstock cutting is formed in the parenchyma. From this vascular tissue, through which nutrients and water are translocated, proceeds to differentiate. The stage before the vascular connection is formed is very critical and the success or failure of grafting is largely determined here. Should the scion bud start growth and become exposed to conditions under which moisture is lost more rapidly than it can be replaced from the rootstock, it will succumb. Sufficient vas= cular tissue must therefore be developed as quickly as possible.

Simultaneously with the formation of callus tissue, root initiation takes place near the bottom of the rootstock cutting which is preparing itself for its physiological role of water and nutrient uptake. Although water is absorbed by the cutting itself, roots are necessary to replace significant amounts lost by the developing scion. The first priority is therefore the formation of a vascular tissue connection through the graft union. Concurrent development of a root system by the rootstock followed by bud break of the scion must take place to produce a successfully grafted plant. These processes often take place in the wrong sequence, or worse still, not enough paren= chyma tissue is formed to ensure a successful vascular connection. This is a major problem with certain rootstocks which do not readily form callus tissue at the apical cut surface.

To promote metabolic processes taking place during the abovementioned chain of events, energy as well as basic structural components are needed. Energy is de= rived from the breakdown of carbohydrate reserves to simpler components, which are further broken down in the respiratory chain with the simultaneous release of energy-rich compounds. Complex carbohydrates and proteins, when broken down, provide sugars and amino acids, which together with certain elements serve as a source of basic structural components for the new growth.

The scion and rootstock cuttings are almost entirely dependent on their stored reserves until such time as the roots commence uptake of nutrients and the leaves start pro= duction of carbohydrates. Callus tissue exposed to light can develop chlorophyll and thus photosynthesise, but in practice the period during which this can occur is only after removal of the grafts from the callusing box and before a cork cambium is formed in the callus.

There is very little knowledge available in respect of the role of reserves during the process of grafting. The main purpose of this study was to trace the accumulation of some of these reserves in the mother vine; their possible utilisation in the graft combination as well as in cuttings which are being rooted.

Such knowledge will lead to a better understanding of the role played by reserve components during the course of vine propagation.

SECTION 2

LITERATURE

2.1 Changes during development of the shoot of the grapevine

The macroscopic development of the vine shoot starts at bud break, and proceeds through several anatomical and physiological stages until winter, when it becomes the mature cane. Initially the shoot serves as a pathway for the transport of nutrients from the place of storage in winter to the newly developing parts, and at an early stage the shoot even produces carbohydrates through photosynthesis. Later is transports carbohydrates from the leaves where they are synthesised to the developing bunch and the shoot apex, and still later from the leaves to the trunk and roots where the carbohydrate reserves had been depleted earlier in the season. During the latter half of the season the shoot starts fulfilling its role as a storage organ, which stores nutrients for the growth of the shoots during the following year. Throughout the season the shoot also has a structural function, in that it spreads out the leaves for maximum exposure to sunlight. Equally important is that the cane is endowed with the new growth organs of the following season which take the form of apical meristems encapsulated in bud scales. A knowledge of the develop= mental history of the canes will greatly assist in the selection of suitable mature canes for cuttings.

2.1.1 Changes in carbohydrates

Deciduous fruit plants are totally dependent on their own carbohydrate reserves for their initial requirements after growth starts in spring. These reserves are stored in different forms such as reducing sugars, non-reducing sugars, starch and, according to some research workers, as part of the hemicellulose fraction. Winkler and Williams (1938) could find no evidence that hemicellulose could serve as a reserve carbohydrate, but Afrikjan, Marutjan and Saakjan (1954, according to Eifert, Panczel & Eifert, 1961) found that a hemicellulose-sugar interconversion could take place. In apple trees part of the hemicellulose does serve as a reserve carbohydrate (Priestley, 1960). According to Priestley (1960) this is because hemicellulose is not a homogeneous component. Part of it can be hydrolysed and used as a reserve carbohydrate.

The term reserve carbohydrates therefore implies those carbohydrate fractions which are available for use in respiration or translocation to areas of utilisation, when new growth starts in spring. The term available carbohydrates is also used to denote these carbohydrates and means the same.

In the vine the roots, especially the thin roots, have the highest starch concentration of all the plant parts and is the main source of reserve carbohydrates when new growth starts (Winkler & Williams, 1945; Weaver, 1976), but the above-ground parts of the plant also contribute (Winkler and Williams, 1945; Eifert et al., (1961). In this respect the vine shows similarity to other deciduous fruit trees. In young apricot trees (Bennett, 1924), young apple trees grown in pots (Stassen, 1973), and young peach trees grown in pots (Stassen 1980), the roots were also shown to be the most important storage organs for starch.

Part of the decrease in carbohydrates in the vine at the start of growth in spring is the result of the use thereof in respiration, in preparation of the plant for growth (Eifert et al., 1961), while the remainder is used in the initial growth of shoots.

Very little work concerning the chemical composition of the shoot has been done thus far. Weaver and McCune (1960) found that the sugar concentration of the shoot base expressed per unit of dry mass, decreased from a sampling on 1 July (Northern hemisphere, NH) when the berries were 12-15 mm in diameter until cropping, and after that increased again. Starch content was very low on the first date, increased until cropping, and subsequently decreased again.

Winkler and Williams (1945) expressed sugar and starch analyses on two bases. On a sugar-and starch-free residual dry mass basis, the basal region of the young shoot, early in the season, showed a peak of reducing sugars which decreased rapidly. The concentrations of sucrose and starch at this stage were very low. These same re= sults expressed as percentage of water content (which is very similar to fresh mass at this stage) did not show the prominent peak in reducing sugars. These results show that, in the case of such young material with a low dry mass content, of which any increase in dry mass can be relatively large, the use of dry mass as a basis for expression of results is difficult to interpret, and that fresh mass or total con= tent should preferably be used.

The most dramatice change in the shoot is the accumulation of carbohydrates, es= pecially starch, during summer and autumn. This accumulation starts before or at the time of cropping and also coincides with the time when shoot growth decreases in relation to existing leaf area (Winkler and Williams, 1945). Eifert et al. (1961) noted that the shoots started discolouring to yellow when carbohydrate accumulation commenced.

Starch concentration of dry mass reaches a peak in autumn, decreases as it gets colder in winter, and reaches a second lower peak shortly before or at the start of bud break. Sugar concentration on the same basis reaches a peak in late summer, decreases as the starch increases up to its peak in autumn, and starts increasing rapidly when starch breakdown occurs after the peak in autumn (Eifert et al., 1961). It reaches a peak at the starch low in winter, and a low at the second starch peak. This reverse fluctuation in starch and sugar content is a typical pattern in vine canes during the cold winter period and is caused by sugar-starch interconversion (Winkler & Williams, 1945; Eifert et al., 1961). Eifert et al. (1961), Koleda (1962 and 1964) and Kondo and Pudrikova (1969) found the highest sugar concentration in the cane during the coldest period in winter. The highest sugar values coincide with the period of maximum cold resistance of the vine (Koleda, 1964; Kondo & Pudrikova, 1969; Cernomorec, 1969). Winkler and Williams (1945) found that the starch-to-sugar conversion was more marked in the phloem than in the xylem of the cane.

The time difference between the two starch peaks varies according to the length and cold intensity of the winter. In the work of Eifert et al. (1961) done in Hungary, the two maxima were five months apart, whereas Winkler and Williams (1945), in research done in California, found the period to be just over four months. Bernstein and Klein (1957), working under warm winter conditions in the Jordan Valley, established that the period between the two starch peaks was just over three months. They could, in contrast with the other authors, not establish a decrease in sugar with the second starch maximum, a phenomenon which they ascribed to the warm winter conditions.

The hemicellulose content in the developing shoot reaches a high value at a very early stage. Winkler and Williams (1945) found nearly maximal content of hemi= cellulose in the bark and wood of the basal parts of Carignane shoots respectively at the beginning and end of May (NH). These results were expressed on a sugar-and starch-free dry mass basis. During winter there was no significant change. Cernomorec (1969) recorded the maximum amount of hemicellulose in the shoots in October (NH) and this coincided with the highest starch content.

Respiration must continue during winter in order to keep the life processes active. In Carignane, Winkler and Williams (1945) measured an average decrease of 0, 5% in the sugar plus starch in the wood of the canes and trunk monthly during the dormant season. Although the phloem may be effectively blocked during the dormant season (Esau, 1948), there is the possibility that some translocation may take place in the xylem, and that the above-ground parts may be supplied from the roots. Hardy and Possingham (1968) detected glucose and fructose in the bleeding sap of vines and came to the conclusion that certain parts of the xylem may have higher concentrations of sugar than the bleeding sap itself. Winkler and Williams (1945) found a larger depletion in the carbohydrate content in the roots than in the above-ground parts, and this might indicate some translocation from the roots to the above-ground parts.

2.1.2 Changes in nitrogen fractions

Deciduous fruit trees are dependent on nitrogenous compounds which have been stored in the plant for their initial nitrogen requirements (Gardner, Bradford & Hooker, 1952). Hill-Cottingham and Lloyd-Jones (1975) found that early spring growth of apple trees in pots was almost entirely dependent on the N status of the tree.

In Thompson Seedless vines (Nassar & Kliewer, 1966) and in apple trees cultivar M II which were grown in pots (Oland, 1959), a large part of the nitrogen reserves are stored in the form of soluble free amino acids, of which arginine comprises the largest part. In work done over a full year on Thompson Seedless, Kliewer (1967) found that soluble nitrogen in one-year old or older parts, comprised 46 to 65 percent of total nitrogen in the roots and 20 to 47 percent in the woody tissues. The percentage soluble nitrogen in the roots and woody tissues was highest during the winter, and reached a peak at the start of bud break. Oland (1959) in studies on young M II apple trees in pots, came to the conclusion that breakdown of in= soluble proteins to amino acids can explain the increase in soluble nitrogen. Kliewer (1967) also came to this conclusion.

Nassar and Kliewer (1966) analysed the wood and bark portions of shoots of Thompson Seedless on four dates namely 30 June, 18 August, 28 September and 1 November (NH) for soluble amino acids. Expressed per fresh mass, the bark showed the lowest value on 18 August, with slightly higher values on the other dates. The wood, however, had about 3 times as much amino acids on the last two dates compared with the first two. Arginine in the wood showed a spectacular increase from 13 μ mole / 100 g fresh mass on 18 August to 590 μ mole / 100 g on 1 November.

The accumulation of carbohydrates during late summer and autumn coincides with the accumulation of nitrogenous compounds. After the rate of shoot growth de= creases and the crop is removed from the plant, the demand for nitrogenous compounds wanes and there is an increase in nitrogen in the different plant parts, including the shoots (Kliewer, 1967). Nitrate, the main form in which nitrogen is absorbed by the roots, is rapidly transformed to NH_4^+ which is then incorporated into amino acids (Grasmanis & Nicholas, 1967; Kirby, 1974), carbohydrates, which are abundant at this time, providing the basic framework for amino acid synthesis (Kliewer, 1967). According to Gardner et al. (1952) amino acid synthesis takes place for the most part in the chloroplasts of the leaf mesophyll cells. From here the amino acids are translocated throughout the plant.

As the leaves senesce, nitrogenous compounds migrate to the shoots. Nassar and Kliewer (1966) showed a decrease per fresh mass in several free amino acids in the leaf blade of Thompson Seedless grapevines from the time of initiation of leaf senescence at the end of September, to a second sampling on 1 November. During this time there was also a rapid accumulation of arginine in the wood of the shoots. This result is in accordance with that of Alexander (1957) who, in work done on Sultana vines, came to the conclusion that 30 percent of the nitrogen lost from the leaves after it had started decreasing in March (Southern Hemisphere) moved back to the shoots, while 70 percent of the loss was caused by leaf fall. Similar translocation of nitrogen from the leaves to the shoots of apples was found by Karmarkar (1934), Murneek (1942) and Oland (1959, 1963).

2.2 Cuttings for propagation

When canes are cut during winter for propagation, this material is in a state of dormancy. Furthermore, the content of reserve carbohydrates and nitrogenous compounds, as well as the relative amounts of the carbohydrate fractions and the nitrogen fractions, can vary according to the time of cutting. It will also be influenced by the history of the growth and nutrition of the vine, especially during the preceding season of growth.

At the time when the cutting is made it has developed its maximal content of car= bohydrates, nitrogenous compounds and elements which, unless augmented from external sources, can only remain constant or decrease. The extent of the loss will depend on the time which elapses from the time of cutting until the cutting or grafted combination starts growing, as well as on external conditions. Carbohy= drates in the form of reducing sugars are the substrate for the formation of high energy compounds such as ATP by the processes of glycolysis, the pentose shunt, the tricaboxylic acid cycle and oxidative phosphorylation. Except for the possibi= lity of leaching or absorption by micro-organisms there is no reason why nitrogen and other elements should be lost.

Therefore, until the cutting or grafted combination has formed leaves which can produce carbohydrates through photosynthesis and roots which can absorb nutrients, the cutting is almost entirely dependent on its own reserves to bring about all the anatomical and physiological changes which are necessary for the formation of these new organs.

2.2.1 Changes in cuttings during storage

Respiration in the vine and in the cutting proceeds throughout the dormant period, even at very low temperatures. Reuther, Cheng and Schneider (1971) detected a release of CO_2 by cuttings stored at $-2^{o}C$. They established that, for cuttings of Riesling and rootstock 5 C taken monthly from November to February (NH) and stored until 19 March, the best sugar-starch conservation was obtained at a storage temperature of $0^{o}C$ as compared to 4^{o} and $10^{o}C$. Between the latter two there was very little difference in sugar-starch loss. Cutting and storage at $0^{o}C$ from any of the months November to February also gave the best bud break compared to storage at the other two temperatures. Cutting in November and storage at $0^{o}C$, $4^{o}C$ and $10^{o}C$ gave bud break percentages of 94, 67 and 48 respectively. The poorer bud break was ascribed by the authors not only to lower carbohydrate content after storage, but also to insufficient cold induction at the higher storage tempera= tures.

Respiration was very dependent on temperature, being promoted by higher tempera= tures. Moving cuttings from a storage temperature of $-2^{o}C$ to $18^{o}C$ resulted in a short, sharp increase lasting about 12 hours, levelling out at a rate about four times as high as that at $-2^{o}C$ (Reuther et al., 1971).

A second starch maximum also develops in cuttings under cold storage conditions (Eifert et al., 1961; Reuther et al., 1971). Reuther et al. (1971) ascribed this second starch maximum, in spite of constant low temperature conditions, to an autonomous regulation mechanism which, apart from temperature, plays a role in the sugar-starch interconversion.

2.2.2 Initiation of growth

Certain internal developments must take place before bud break, the initiation of adventitious roots, or before the formation of callus can commence. The cutting taken late in winter is in a condition of quiescence, but under suitable conditions of temperature will undergo a chain of anatomical and physiological processes leading to bud break, root growth and callus formation.

The first anatomical change which is visible is the reactivation of the phloem when the callose deposits on the sieve plates and sieve areas disappear. There is no definite evidence that disapperance of the callose is related to the position of the buds as is the case with cambial development. The initiation of cambial activity in the cane on the vine usually starts beneath the buds and then progresses down= wards and around the cane (Esau, 1948).

According to Esau (1948), a certain amount of bud development is necessary for cambial development (established vines). It is, however, possible to obtain callus formation by the cambium on an internodal piece of detached cane on which no bud is present. This also holds good for longer cuttings with nodes where the buds have been removed.

Skene (1971), in an investigation into the release of sugars into the xylem sap in cane segments of Cabernet Sauvignon came to the conclusion that some cambial development was necessary before the addition of indole acetic acid (IAA) could stimulate the release of sugars. Cambial development was stimulated by the presence of a growing bud or by pre-incubation of only the internode segment in an IAA solution. According to Skene (1971) starch degradation may be caused by increasing metabolic requirements of an actively growing cambium.

Along with the activation of the cambium in the cutting, it can under suitable con= ditions initiate adventitious roots at the base, and form callus tissue at any wound. Adventitious roots develop from meristematic regions near the surface of the cuttings (Weaver, 1976). Shimoya, Gomide and Fortes (1971) found root initiation in the rootstock IAC - 313 in the vascular ray extensions. In grafting a scion onto a rootstock cutting, the formation of callus at the bottom of the scion and at the top of the rootstock is essential in order to establish a bridge of callus tissue in which cambium and vascular tissues can later differentiate. Without this callus the graft union cannot be formed (Orffer, 1956; Winkler, Cook, Kliewer & Lider, 1974; Hartman & Kester, 1975). In the grapevine cutting, callus at the apex is mostly formed by the cambial zone, while at the base the cambial zone, phloem rays and phloem parenchyma are the tissues most active in producing callus (Orffer, 1956).

The ease with which different cultivars form roots and callus varies, and much research work has been done in order to find means of stimulating the processes in order to obtain a higher percentage take of grafts or rooting of cuttings. Only certain ways of stimulating rooting and callusing which are of importance for this study will be discussed (section 2.2.4).

2.2.3 Rooting and callusing of Jacquez and Salt Creek cuttings

Jacquez (*Vitis aestivalis* - *V. cinerea* - *V. vinifera*; Galet, 1956) and Salt Creek (Ramsey) (V. champini; Winkler *et al.*, 1974) are at present the two most im= portant rootstocks for table grapes in the Western Cape.

Jacquez has good affinity generally, induces moderate growth and good fruit-set characteristics in the scion cultivar, and is very well adapted to the more fertile soils such as decomposed granite or alluvial black soils along the rivers. It forms callus fairly well, roots reasonably well, and is preferred by nurserymen to Salt Creek for bench grafting.

Salt Creek also has a good affinity generally but affinity problems with Hanepoot, Alphonse Lavallée and Barlinka related to virus infection have been encountered. It is better adapted to the less fertile sandy or stony soils where its vigour gives it an advantage over Jacquez. Because of poor callusing at the apex of the cutting, Salt Creek does not do well in bench-grafting, and the percentage take is often very low. However, Bosman (1976) reported that very early collection of Salt Creek cuttings (end of April to first week in May) (SH), combined with soaking in water, improved callusing ability at the apex.

Apart from increasing the price of the plant, the scarcity of table grapes grafted on Salt Creek results in many producers using rootstocks which graft more easily, but are not as suited to their soil conditions. This causes them to lose out on production throughout the life of the vine, as well as shortening the life-span of the vine.

Although there are reports to the contrary, Salt Creek roots reasonably well, if canes are collected during autumn (end of April- beginning of May) or in late winter (Bosman, 1976).

2.2.4 Stimulation of callusing and rooting by auxins

Endogenous auxins play an important role in many parts of the plant, including stimulation of cell division, thus being able to promote cambium development and root initiation. Auxins are very effective in stimulating root formation (Hartman & Kester, 1975; Weaver, 1972).

According to Weaver (1972), indole acetic acid (IAA) is very unstable in plants, and therefore the synthetic auxin, indole butyric acid (IBA) is frequently used. IBA is destroyed relatively slowly by auxin-destroying enzymes. It translocates poorly and is therefore retained near the site of application. It has a weak auxin activity.

Many researchers have reported on the effective stimulation of rooting in grape cuttings by the use of IBA (Tizio, 1962; Julliard, 1964; Almela Pons & Tizio, 1965; Antagnozzi, Cartechini & Preziosi, 1968; Schenk, 1969; Stepanova, 1969). Alley (1962), Goussard (1972), Goussard and Van der Merwe (1973), Alley and Petersen (1977) and Alley (1979) all reported stimulation of rooting in Salt Creek by IBA.

2.2.5 Changes in the cuttings during shoot and root growth

Buttrose (1966) studied the utilisation of carbohydrates during the initial growth of Sultana cuttings after they had first been given bottom heat treatment at 25°C for four weeks to develop roots, while the top was kept at 4°C. After this the plants were planted in a glasshouse. Two-node and one-node cuttings as well as one-node cuttings which were shaded in the glasshouse were used. (The last treatment will not be discussed). Samples were taken before rooting, at the time of planting out, and 25, 39, 53 and 67 days after planting out.

The whole plants lost dry mass until 25 days after planting out, the one-node cuttings losing the largest percentage of their initial dry mass, but subsequently showing the biggest proportional increased based on initial dry mass. The final dry mass of roots plus shoots of the 2-node cuttings was higher than that of the 1-node cuttings. The cuttings alone showed the maximum loss from their initial dry mass (20-22%) 39 days after planting. Starch in the cutting decreased from 12% o a little below 2% of initial cane dry mass 39 days after planting, and after 53 days it started increasing again. The two-node cuttings showed a slightly slower decrease and higher increase rate in starch concentration compared to the one-node cutting. Sugar stayed fairly constant over the period, decreasing from about 3 to 2% while in the rooting box, regaining part of this loss during the first 25 days after planting out.

Hemicellulose concentration decreased between the initial sampling before rooting and 39 days after planting. The two-node and one-node cuttings showed a drop in the percentage expressed on initial dry mass from 17,8 and 17,4 to 13,3 and 11,9 respectively. These losses represented 25,2 and 31,6% of the respective initial values.

Schaefer (1978) studied the changes in sugar, starch, total N, protein N and soluble N in three graft combinations during the season after grafting. The results were basically the same for the three combinations, so it would suffice to discuss those for the Müller-Thurgau/193 G x Rip 1 G combination. During callusing (30 March to 20 April) sugar + starch increased in the rootstock cutting and decreased in the scion. From the start of hardening off on 20 April until 23 June, sugar + starch decreased constantly, thereafter increasing to peak levels on 12 October, just before the start of wood ripening. After the peak levels, the concentration de= creased for three weeks, followed by small increases during the subsequent 8 weeks. By this time the leaves had already fallen. Sugar + starch in the shoots showed the following changes: an increase to a peak level on 12 October, a decrease, and an increase until 31 January. The roots showed two peak levels in sugar + starch; firstly, at the same stage as the other parts on 12 October, and secondly, 6 weeks later on 25 November.

Sugar in all parts remained at fairly even low levels throughout the season until 2 November when it increased at the cost of starch. Only in the canes did sugar concentration ever exceed that of starch, and that was during a short time in winter. In this case there was also an apparent conversion of sugar to starch from 28

December to 31 January.

In the cutting and scion, total N and soluble N showed a decrease during the three weeks callusing period until hardening off commenced. Soluble N concentration of fresh mass at this stage comprised roughly one-quarter of the total N.

Hosoi, Machida and Yoshida (1972) studied the changes in N and carbohydrates in cuttings of Delaware grapes during the first 80 days of development. There was a decrease in dry matter until 60 days after planting in both the top and lower half of the cutting. Other results were expressed per dry mass. Total sugar decreased quickly, and reached a low 40 days after planting after which there was a slow increase. Starch decreased slowly (initially sugar was slightly higher than starch). Little difference was found in the content of sugars and starch between the upper and lower halves of the cuttings.

Soluble N gradually decreased in both upper and lower halves of the cuttings, while insoluble N only decreased markedly in the upper half of the stem. After 80 days the roots contained only 3% of thc total N in the plant, the rest being equally distributed between the shoots, upper half of the cuttings and lower half of the cuttings.

Obink, Alexander and Possingham (1973) observed that up to 65% of the N and up to 48% of the K in dormant vine canes can be re-utilised for the formation of new shoots and roots. In plants (cuttings) which were grown without N fertiliser for 10 weeks, the shoots contained 38,6% and the roots 24,5% of the total N in the plant. More N was therefore translocated to the shoots than to the roots. In plants (cuttings) grown without K fertiliser the shoots contained 40, 7% and the roots 8, 5% of the K in the plant after 10 weeks growth. More than 4, 5 times the amount of K was therefore translocated to the shoots compared to the roots. The K reserves in the cuttings were sufficient for 10 weeks of growth, but severe K deficiency symptoms had already developed at that stage.

Hosoi, Machida and Kurogouchi (1971) studied changes in macro-elements in plants developing from cuttings in Delaware grapes during the first 100 days of growth in sand culture. More N, P, K, Ca and Mg was translocated to the new shoots than to the roots. One hundred days after planting 43 N, 37% P, 49% K, 35% Ca and 50% Mg had been translocated from the stem to the newly developed shoots and roots.

SECTION 3

GENERAL PROCEDURES

3.1 Material

For all experiments sample cuttings were taken from Jacquez clone 1/3/11 and Salt Creek clone 5/19/5 mother vines, which were four years or older and growing on an oakleaf soil on the Bien Donné Experimental farm near Groot Drakenstein. Both clones tested free of leafroll, fleck and the fanleaf complex. Waltham Cross clone 22 scions were sampled in a vineyard on the Bellevue Experimental farm in Paarl, where they were growing on a yellow clovelly soil. This clone, in spite of having shown leafroll and fleck symptoms during indexing, was the best clone in the Waltham Cross selection program (Anon., 1971).

The cuttings or scions were recut to provide a fresh surface before callusing or planting. These cuts were made at a right angle to the length of the cutting or scion. Cuttings for callusing were recut 1 cm below the bottom node and 5 cm above the top node, and disbudded except for the basal bud. Wounds made by removal of a bud were singed with a soldering iron to prevent callus formation at these wounds. Scions were recut with one bud, 1 cm above the bud and 5 cm below the bud. Prior to placing the cuttings or scions into callusing bags they were dipped in captab (5 000 mg/l). Cuttings for rooting were recut 1 cm above the top bud and 1 cm below the bottom bud. All buds except the apical and basal ones were removed.

As cuts were made in relation to buds, cuttings were not all of the same length. Cuttings for callusing varied between 20 and 25 cm in length and from 6 to 10 mm in diameter. Those for rooting varied between 25 and 30 cm in length and from 3 to 6 mm in diameter.

3.2 Preparation of samples for analysis

Samples were first washed in a 0,15% "Teepol" solution, rinsed twice with tap water and twice with distilled water (Beyers, 1962) and allowed to dry at the sur= face. After their fresh mass had been determined, they were put into a blast freezer at -25°C as soon as possible and kept there until freeze-drying. After drying, the dry mass of the sample was determined. This dry sample was then ground twice, the second time to pass through a 0,4 mm sieve. The ground powder was stored in screw-top glass jars at -25°C until analysed. Before analysis, the samples were again dried in a vacuum oven at 30°C for three hours.

3.3 Analytical methods

3.3.1 Analytical methods for carbohydrates

3.3.1.1 Alcohol-soluble sugars

The method used was adapted from methods applied by Marais (1965), Gaines (1973) and Stassen (1973).

Sugars were extracted from the sample with 80% ethanol and after evaporation of the ethanol, the extract was cleared of proteins and pigments by the addition of balanced volumes of $Ba(OH)_2$ and $ZnSO_4$ according to the method used by Marais (1969). Di-and oligo-saccharides in the extract were acid-hydrolysed, followed by neutralisation with alkali. Any remaining colour was removed with deactivated charcoal. Details of the method are described in the Appendix.

3.3.1.2 Starch

Starch in the alcohol-extracted residue obtained in 3.3.1.1 was extracted by the taka-diastase method described by Marais (1969). This method entails incubation of the sample with taka-diastase, clearing the filtrate of protein and pigments with $Ba(OH)_2$ and $ZnSO_4$, followed by an acid hydrolysis, neutralisation and decolouration with deactivated charcoal. The method is described in full in the Appendix.

3.3.1.3 Hemicellulose

Hemicellulose was extracted from the starch-free residue obtained in section 3.3.1.2 by acid hydrolysis as used by Stassen (1973). This method was adapted from re= search work by Turner (1969) and is basically the same as that of Winkler and Williams (1938) except for the use of a higher HCl concentration (0,7 mole/dm^3) for hydrolysis. Hydrolysis was followed by filtration, neutralisation and clearing of the filtrate of proteins and pigments with $Ba(OH)_2$ and $ZnSO_4$. The detailed method is presented in the Appendix.

3.3.1.4 Determination of reducing sugars

Reducing sugars in the samples were determined on a Technicon Auto-Analyser, using a slightly modified version of the ferricyanide method of Bowen and Nonaka (1967). Gluclose was used as a standard. The method is described in detail in the Appendix.

3.3.2 Analytical methods for nitrogenous fractions

For total N ca. 0,2 g dry sample was accurately weighed and digested in a 50 ml Kjeldahl flask with 3 ml conc. H_2SO_4 plus a knife-tip selenium mixture (Merck). After making up to volume, ammonia was determined by the Technicon Auto-Analyser II industrial method no. 98-70W.

Soluble nitrogen was obtained by extracting ca. 2 g dry sample which was accurately weighed with 50 ml 80% ethanol for 15 hours at room temperature while shaking. The filtrate was washed into a 100 ml Kjeldahl flask, H_2SO_4 and selenium mixture as for total N added, and the flask put onto a micro Kjeldahl digestion unit. After evaporation of the excess ethanol and water, digestion commenced. The rest of the method was the same as for total N.

3.3.3 Analytical methods for elements

Approximately 2 g dry sample, accurately weighed, was dry-ashed. After making up to volume, phosphorus in the solution obtained was determined by the Technicon Auto-Analyser II industrial method no 94-70W/tentative. K, Ca and Mg was de= termined by atomic absorption (Mc Bride, 1967).

3.4 Procedure for callusing cuttings and scoring of callus

The bag for callusing the cuttings was prepared as follows (Fig. 1).

A 410 x 610 mm polythene bag was laid on its side and a 6 mm foam rubber pad, which had been wetted in water containing captab (5 000 mg/1) was placed on the bottom. About 10 cm from the top and bottom of the pad polystyrene strips of 25 x 25 x 280 mm were placed diagonally. Two bent wires were used to support the top of the polythene bag and the cuttings were laid lengthwise on these strips. The bag was then tightly closed.

Temperature in the callusing room was maintained at 25°C, while humidity was kept at 95% by means of a humidifier. The room was kept dark.

For scoring of the callus the cut end was held facing the scorer with the bud, or the position where the bud had been removed, at the top. The cut surface was then divided into imaginary quarters (Fig. 2a). Each quarter was given a mark out of 4 for the extent of development of the ring of callus (Fig. 2b). The amount of callus in this area was then scored out of 3, with 1 for a thin strip of callus, 2 for a wider strip and 3 for a very complete covering from the outer diameter of the cutting until close to the middle (Fig. 2c).

Callus was therefore scored out of a total of 28. A score of 20 was, for instance, obtained for a thin continuous callus ring while a score of 28 was obtained for a broad continuous callus ring.

3.5 Procedure for planting cuttings for rooting

In order to be able to remove rooted plants from the soil with as many intact roots as possible, a special procedure was adopted. This was used for cuttings rooted in sections 4, 10 and 11.

A plastered brick trough 75 cm deep and 1 m wide, filled with a sandy soil (3% clay) (section 4, 10) or acid washed sand (section 11), and standing in the open was used. The trough was divided into sections by means of 100 μ polythene sheets. A temporary hardboard support was used while each section was being filled (Fig. 3a). The rooted plants were washed out by means of a garden hose (Fig. 3b), and afterwards the sand was again filled in, in front of the exposed polythene sheet (Fig. 3c). In each section one row of 10 cuttings was planted.

3.6 Expression of results

There are several possible bases on which results can be expressed, and in these studies it was found necessary to use different ways, depending on the type of experiment.

3.6.1 Dry mass

3.6.1.1 Percentage of dry mass

This is the most popular method used by agricultural researchers for expression of analytical results of this nature. The reason for this is the accuracy with which dry mass can be determined. It was found useful for comparing results on different sampling dates over a period of a few months up to one year, such as in sections 4, 5 and 10.

3.6.1.2 Amount per dry mass

The amount of CO_2 evolved in cut regions (sections 7, 12) was expressed in ng per g dry mass per second.

3.6.1.3 Percentage of initial dry mass

It was found necessary to adopt this method of expressing analytical results in studies in which the cuttings initially had a certain dry mass, but during the pro= cess of callusing, rooting or storage for a period of time, some of the substances making out part of the dry mass were lost, for example the loss of sugar and starch. Because of this loss it is possible that an element such as Mg may show an increase when expressed as percentage of dry mass when actually there had been no real increase in Mg. This also holds true for a carbohydrate fraction such as hemi= cellulose. In order to nullify this effect, results were expressed as percentage of initial dry mass. This was done by determining the final dry mass as percentage of the initial fresh mass and using the following correction factor.

$$\frac{\text{Dry mass as \% of initial fresh mass for the later sampling date}}{\text{Dry mass as \% of fresh mass of initial sample}}$$

Multiplying this factor with the analytical result therefore gave the results as percentage of initial dry mass.

In section 6, where division into good and poor callusing was done only after callusing, the initial percentage dry mass of the cuttings could not be determined separately and one correction factor was therefore used for good and poor callusing. As it turned out, the loss in sugar, starch and hemicellulose which to a large extent contributed to the loss in dry mass was very much the same in the good and poor callusing samples and therefore the error made in using a single factor was insignificant.

Likewise, although the initial fresh mass of each part of the cutting on 2 September was obtained, it was found that the division into four parts could not be repeated accurately enough on the later sampling dates. This was mainly caused by the irregular shape and lengths of the cuttings. The correction factor for each part of the cutting would therefore be more inaccurate than the use of one factor for the whole cutting. As the sugar, starch and hemicellulose lost from the four cutting parts varied very little, the error in using one factor for the whole cutting was small.

Expression of results on the basis of initial dry mass was also adopted for cold storage of cuttings (section 5), callusing at different temperatures (section 8) and callusing of cuttings of different lengths (section 9).

3.6.2 Percentage of fresh mass

In an experiment in which a large change in dry mass occurs, as in section 4, re= sults based on dry mass are difficult to interpret. In this case it was deemed necessary to express results as percentage of fresh mass as well.

3.6.3 Absolute amount (content)

Where development of new plant parts occurs, for instance development of roots, shoots and leaves from the initial cutting, the changes in the new parts have to be followed separately from the cutting (section 10). In order to be able to relate the different plant parts to each other, another basis of expression viz. the absolute amount per plant part, is particularly useful.

This method was also found useful where a loss of a certain fraction such as sugar occurred from a fixed initial amount. It was therefore also used in experi= ments described in sections 6 and 11 to express the changes in the whole cutting during the period of callusing or rooting.

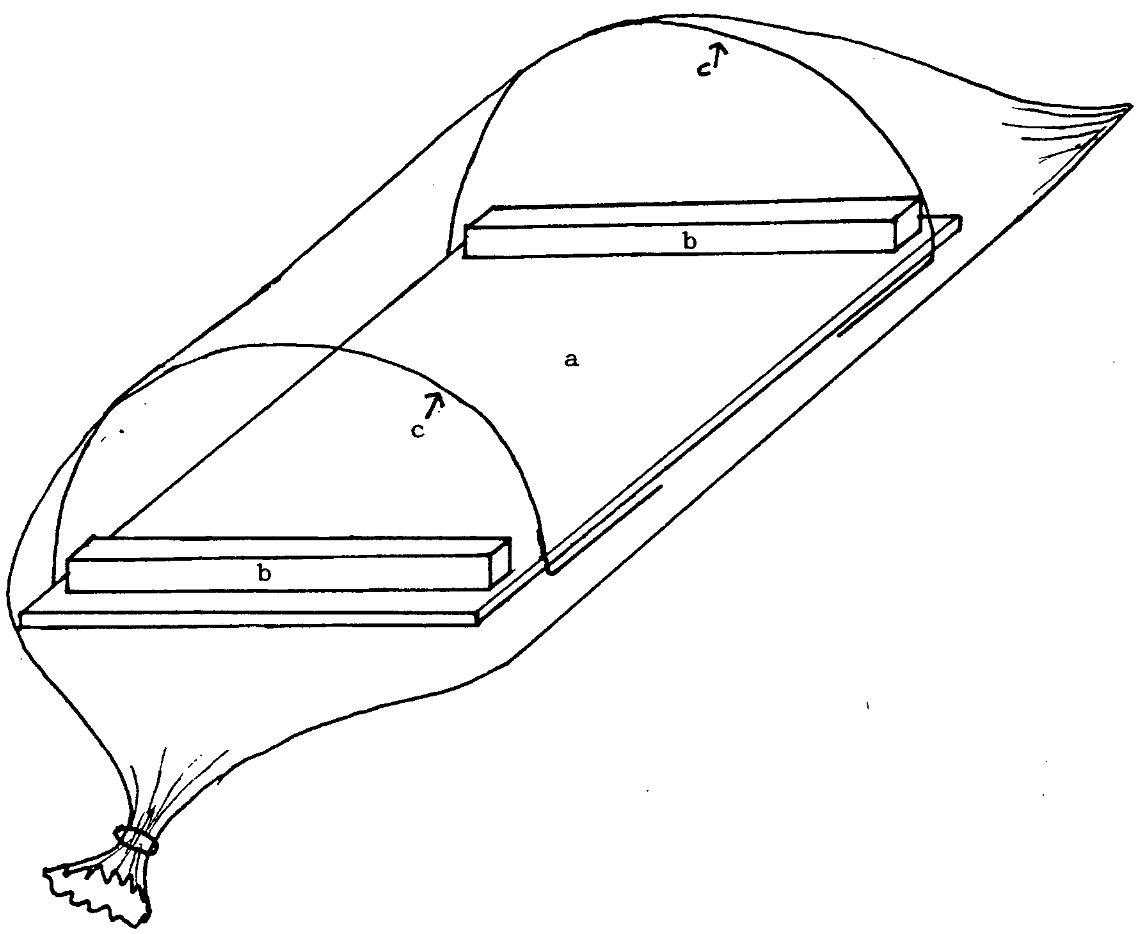


Fig. 1 - Callusing bag containing foam rubber pad (a), polystyrene strips (b) and wire hoops (c)

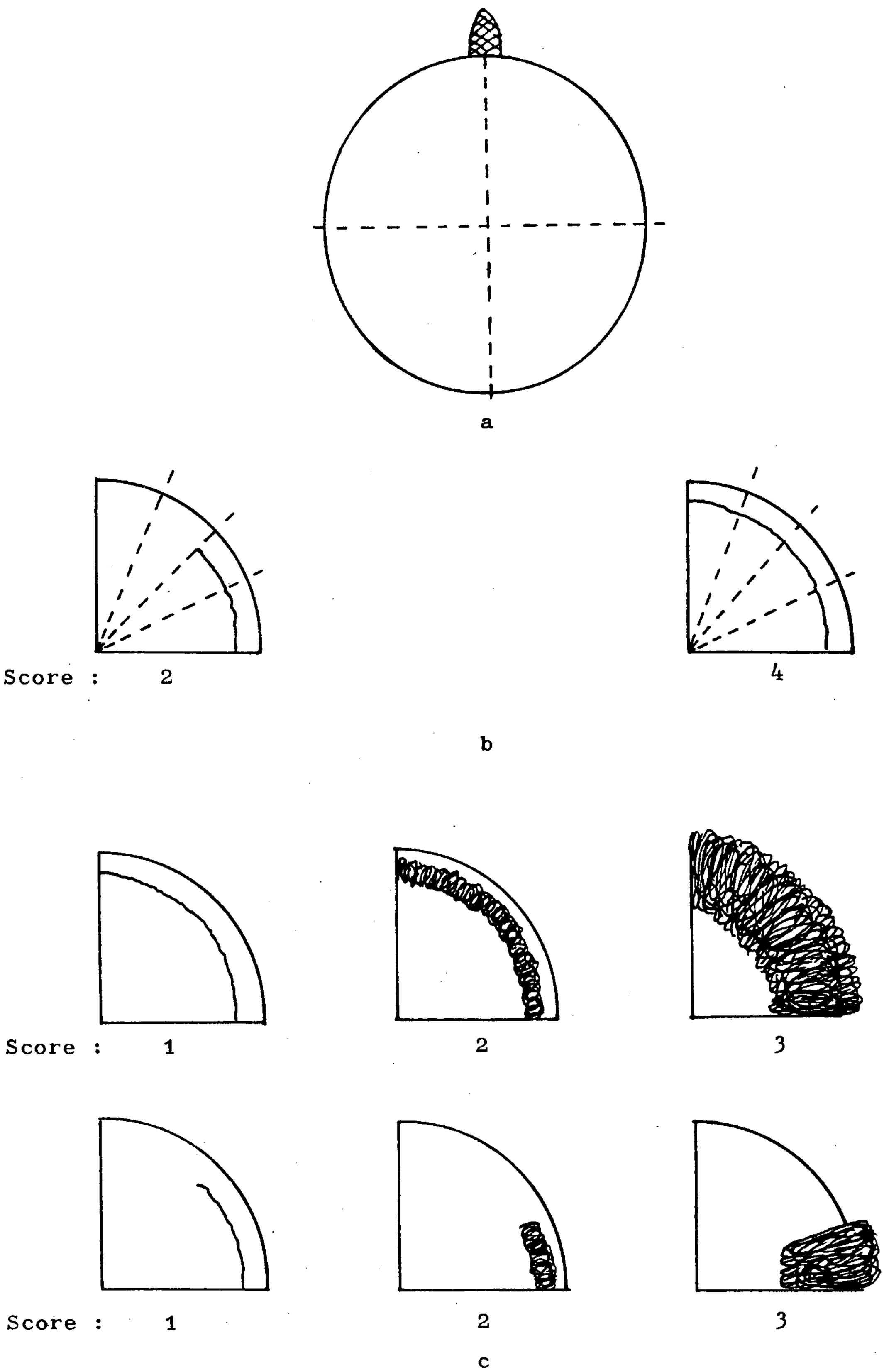


Fig. 2 - (a) Division of the cut surface into quarters

(b) Scoring for portion of cambium where callusing had taken place

(c) Scoring for amount of callus

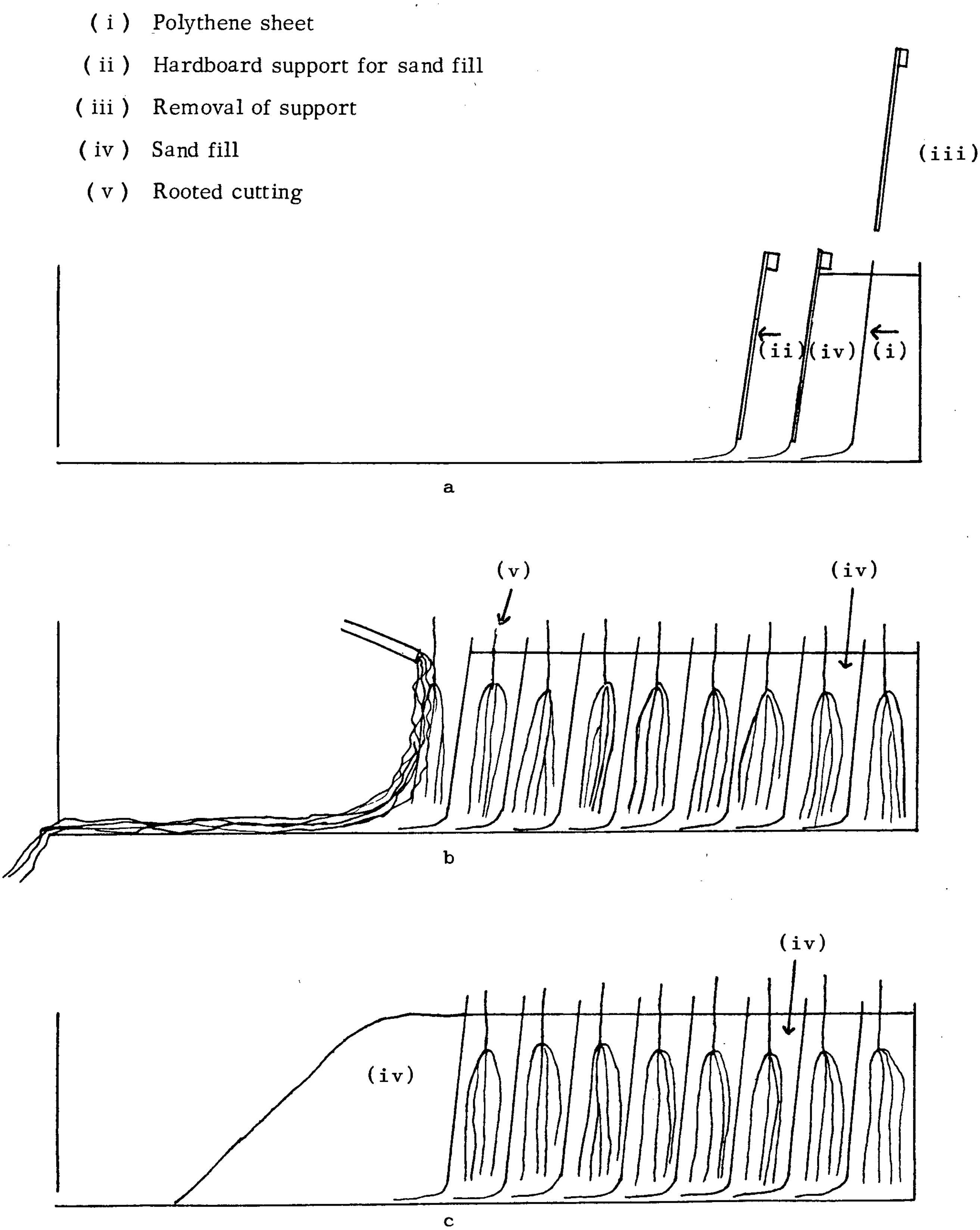


Fig. 3 - (a) Insertion of polythene sheet

(b) Washing out one row of cuttings at a time with water

(c) Replacement of sand in front of polythene sheet

SECTION 4

SEASONAL CHANGES IN CARBOHYDRATES AND NITROGEN FRACTIONS IN SHOOTS (CANES) OF THE MOTHER VINE; REGION FROM WHICH THE CUTTING IS TAKEN AND COMPOSITION OF THE CUTTING ON CALLUSING AND ROOTING

4.1 Introduction

In practice, hardwood cuttings of the grapevine are made during the dormant period when the canes are fully ripe. Cuttings of rootstocks which root readily can be made at any stage during that time, but V. berlandieri and other species which root difficultly must be cut very early or very late during this period for best results (Babó & Mach, 1909). They regarded a small pith coupled with sufficient starch accumulation as the criterium of good physiological ripeness of the cane.

In order to quantify the cutting quality with respect to its callusing and rooting abilities, the seasonal changes in some factors which might affect these were studied for cuttings taken from different regions of the shoot system of the mother vine. According to the literature the most probable factors which might play a role appeared to be the composition of the cuttings especially with regard to starch (Babó & Mach, 1909) or sugar and starch (Eifert, Panczel & Eifert, 1961; Winkler et al., 1974). The cane region from which the cutting is taken is also related to ripeness, the apical region being mostly immature. There is also a general decrease in the diameter of the cane as distance from the base increases.

Cuttings of 6 to 10 mm in diameter, which are mostly used for bench grafting are therefore mostly taken from the older part of the primary cane and the basal secondary canes. The apical region of the primary cane and the rest of the secondary canes mainly produce thin cuttings which are suitable for rooting.

Very little is known about the composition of the different regions of the vine cane and the influence this may have on callusing, root growth and shoot growth. This study had a dual purpose. Firstly, to trace the seasonal changes of certain components in different shoot (cane) parts. Secondly, to determine whether differences exist between the cane regions with regard to callusing, rooting and shoot growth, and if this were so, to determine whether this difference could be related to composition of the cane.

4.2 Procedure

4.2.1 Procedure for the study on seasonal changes

Due to limitations imposed by the quantity of available plants, a formal statis= tical design could not be followed. The statistical analysis was done according to the method advised by a biometrican. The experimental layout was as follows.

Vines of Jacquez and Salt Creek which were spur-pruned close to the soil surface in July 1975, were used. There were 11 sampling dates viz. 1 October, 3 November, 1 December, 2 January, 2 February, 1 March, 1 April, 3 May, 1 June, 1 July and 2 August. On each of these dates ten shoots of medium to long growth were removed from each of 6 randomly selected vines per cultivar. These shoots were sub=divided as follows (Also see Fig. 4).

Basal region of the primary shoot (BRP): Basal metre of the primary shoot
Middle region of the primary shoot (MRP): Second metre of the primary shoot
Apical region of the primary shoot (ARP): Rest of the primary shoot
Basal region secondary shoots (BRS): Secondary shoots on the BRP
Middle region secondary shoots (MRS): Secondary shoots on the MRP
Apical region secondary shoots (ARS): Secondary shoots on the ARP

At the first sampling only the BRP sample was taken, while sampling of the rest was started when available.

All samples were analysed for sugar, starch, hemicellulose, total N and soluble N. Results were expressed as concentration of fresh and dry mass.

4.2.2 Procedure for study on callusing, root and shoot growth

In the same experiment described in 4.2.1, samples were also taken on 2 August for this study. For this purpose, 20 and 10 cuttings were taken from each part for determination of the callusing and rooting ability respectively.

Callusing was done at 25 ^{o}C for 18 days and after this the callus on both ends of the cuttings was scored. For rooting, the cuttings were planted according to the procedure described in section 3.5. Fertiliser (3.1.5 (42) WS) was applied bi - weekly from planting until the end of February at the rate of 5 $g.m^{-2}$. Over= head micro-irrigation was applied whenever necessary.

The cuttings were allowed to grow until 25 July 1977 at which stage they were removed from the sand (section 3.5). The fresh and dry mass of the roots and shoots which had developed were recorded.

The various constituents (sugar, starch, hemicellulose, total N, soluble N) of the samples taken on 2 August in the previous study (section 4.2.1) expressed as concentration of dry mass, were correlated with the callus score, root mass and shoot mass. These three variables were also correlated with the percentage dry mass and the fresh mass of the cuttings used for either callusing or rooting.

4.3 Results and discussion

4.3.1 Seasonal changes

In English terminology the shoot becomes a cane at the time of leaf drop. In order to avoid confusion only the term shoot will be used in this section, but this will refer to the cane during the dormant period.

4.3.1.1 Percentage dry mass

The seasonal changes in percentage dry mass of different shoot regions of Jacquez and Salt Creek vines are presented in Figs 5a, 6a and Appendices 1.1, 2.1.

The percentage dry mass of all shoot regions of Jacquez and Salt Creek showed more or less linear increases from the first sampling until 3 May. During this period the younger shoot regions tended to have lower values. Losses in all regions during May and in some parts during June were followed by increases until 2 August. According to the seasonal changes in the percentage dry mass of the shoots, two distinct periods can be identified, firstly a period of rapid increases until 3 May, and secondly a period of less pronounced changes corresponding with the dormant period.

4.3.1.2 Sugar, starch and hemicellulose

During the first part of the growing season there was a drastic difference in the pattern of graphs based on dry and fresh mass. This can be seen in Figs 5b, c and 6b, c and was caused by the rapid increase in percentage dry mass until 3 May.

Because the results on a fresh mass basis lends itself better to meaningful inter= pretation only the results expressed in this way will be discussed in this section, except where otherwise stated. However, as it is important to be able to relate these results to those of the following experiments where dry mass is used as a basis for expression, results expressed in this way are also presented.

Seasonal changes in sugar concentration of dry and fresh mass in different shoot regions of Jacquez and Salt Creek vines are presented in Figs. 5b, c; 6b, c and Appendices 1.2, 1.3, 2.2, 2.3.

Sugar concentration of fresh mass was low in all shoot parts of Jacquez when they were initially formed. Gradual increases in all parts followed, culminating in slight peaks on 1 March. At this stage the ARP had the highest content (2,41%). During April small decreases in sugar in the primary shoot parts and the BRS were again noticeable. From 1 June a dramatic increase occurred. In fact, the sugar concentration in all parts more than doubled in the two months prior to 2 August.

The sugar concentration of fresh mass in the Salt Creek shoot regions was also initially low, and it remained low until increases occurred during April, which cul= minated in slight peaks on 3 May, two months later than those of Jacquez. At this stage the highest concentration was obtained in the ARS. These later peak levels, compared to those of Jacquez, were undoubtably caused by the longer growing season of Salt Creek. In all Salt Creek shoot regions decreases during May were followed by the same dramatic increases as in Jacquez during June and July. Through= out the season sugar concentration in Salt Creek remained somewhat lower than in Jacquez.

Seasonal changes in starch concentration of dry and fresh mass in different shoot regions of Jacquez and Salt Creek vines are presented in Figs. 7a, b; 8a, b and Appendices 3.1, 3.2, 4.1, 4.2.

The starch concentration of fresh mass in all shoot regions of Jacquez was initially still lower than that of sugar, increasing slowly from 1 December, with fairly large increases during February and March. These increases culminated in rather flat peaks, stretching from 1 April to 1 June, for all parts. During this period the primary shoot regions had the highest values, with a maximum of 5,37% in the BRP on 3 May. During June and July there were considerable losses of starch in all regions, the largest losses being sustained in the primary regions.

The starch concentration of fresh mass in all shoot regions of Salt Creek was also initially lower than the corresponding sugar value. It increased more slowly com= pared with that of Jacquez, real increases only showing from 2 January. The largest increases in all regions took place during March and April, resulting in pronounced peaks on 3 May. The highest values were attained in the primary shoot regions, especially the BRP with 4,63%. During May, June and July there were marked losses of starch in all regions, especially in the primary ones, as was also recorded in Jacquez.

In both Jacquez and Salt Creek the primary shoot regions nearly always showed a higher starch concentration than the secondary shoots. This difference was the largest at the time of the highest starch values. Starch concentration in all shoot regions of Salt Creek remained lower than the corresponding concentration in Jacquez throughout the season.

It is clear that on the basis of sugar and starch concentrations, the period under investigation can be divided into two distinct stages. The first stage started after budbreak and continued until the end of May to early June. During this stage sugar remained at a fairly constant level around 1 - 2%, the excess sugar being converted to starch and other products. The sugar therefore constituted a labile pool from which it was available for conversion to starch as a reserve or for the formation of other products for growth. The second stage which covered the period early June to early August, was characterised by the conversion of starch to sugar, a mechanism that provides protection against low temperatures (Winkler et al., 1974).

The pattern of sugar and starch changes as well as the actual concentration as percentage of dry mass obtained in this study correspond very well with that of Eifert et al. (1961). Under the warmer winter conditions in South Africa, compared to that of Hungary, the starch to sugar conversion did not proceed as far as that found by these authors. This resulted in somewhat higher starch minimum and lower sugar maximum values during the coldest period.

Seasonal changes in sugar + starch concentration of dry and fresh mass in different shoot regions of Jacquez and Salt Creek vines are presented in Figs. 7c, d; 8c, d and Appendices 3.3, 3.4, 4.3, 4.4.

The sugar + starch concentration of fresh mass in both Jacquez and Salt Creek followed very much the same pattern as that of starch up to 1 June, as during this period starch predominated. All shoot regions of Jacquez showed decreases during June, followed in July by small losses in the BRP and MRP, no change in the MRS and increases in the other regions. In Salt Creek, all regions showed increases during June and July, especially the ARP, MRS and ARS.

Seasonal changes in hemicellulose concentration of dry and fresh mass in different shoot regions of Jacquez and Salt Creek vines are presented in Figs. 9a, b; 10a, b and Appendices 5.1, 5.2, 6.1, 6.2.

The hemicellulose concentration of fresh mass in all regions of Jacquez increased very rapidly from initial low values, reaching some of the highest values during the season on 2 February. On this date the BRP and MRP showed the maximum values (11,10 and 11,11% respectively). After this all regions except the ARS showed general small decreases up to 3 May followed by a second series of peak levels on 1 June. During June considerable losses occurred in all regions, es= pecially in the primary shoot. These losses are significant, as it means that at least part of the hemicellulose, which is a structural component, was broken down. Comparison of the starch decreases and sugar increases during this period, shows that the latter cannot be explained solely on the basis of a starch to sugar conversion. The hemicellulose which was lost most probably contri= buted to the sugar increase. The regions of the primary shoot tended to have the higher hemicellulose concentrations throughout the season.

Salt Creek showed less pronounced increases than Jacquez from the initial hemi= cellulose values in all regions, and also reached peak levels on 2 February. The highest value here was attained in the MRS (8,59%). During February all regions showed losses. This was followed by a buildup starting in March, which culmi= nated in second, considerably higher, peak levels on 3 May, a month earlier than the corresponding peaks in Jacquez. The highest value reached at this stage was

11,36% in the ARP. During May and especially during June, all regions of Salt Creek showed losses of hemicellulose. Similarly to Jacquez, sugar increases during June cannot be explained solely on the basis of a loss in starch; there was probably also a conversion of some hemicellulose to sugar.

Winkler and Williams (1938) expressed the hemicellulose content during development of the shoots of Carignane on a sugar + starch - free dry mass basis. They recorded a very rapid increase in hemicellulose concentration in the young shoot, similarly to the results based on dry and fresh mass in the present study. They did not however find the same apparent breakdown of hemicellulose during early winter. If the different bases of expression in the two studies are taken into account the concentrations of hemicellulose recorded by Winkler and Williams during the winter months were somewhat the lower.

In both cultivars, hemicellulose was the first of the three carbohydrate fractions so far discussed, to accumulate, as it is an important structural component. Starch, which is a reserve carbohydrate, started accumulating somewhat later, while sugar only reached high concentrations during the winter months. Seasonal changes of these three components combined as concentration of dry and fresh mass in different shoot regions of Jacquez and Salt Creek vines are presented in Figs. 9c, d; 10c, d and Appendices 5.3, 5.4, 6.3, 6.4.

The graph patterns based on fresh mass obtained here (Figs. 9d, 10d) compare remarkably well with those of the dry mass (Figs. 5a, 6a). This is not sur= prising as these components comprised between 25 and 40% of the dry mass during most of the season (Figs. 9c, 10c). The sugar + starch + hemicellulose increased steadily throughout the growing season in all regions, reaching peak levels on 3 May in Salt Creek and 1 June in Jacquez. The loss after these peaks can possibly be attributed to one or more of the following three reasons:-

- Utilisation in respiration
- Translocation to the roots
- Transformation to other components contributing to the dry mass.

4.3.1.3 Total N and soluble N

Seasonal changes in total N concentration of dry and fresh mass in different shoot regions of Jacquez and Salt Creek vines are presented in Figs. 11a, b; 12a, b and Appendices 7.1, 7.2, 8.1, 8.2.

From initial high values, total N concentration of fresh mass in Jacquez decreased until February and March. Thereafter it again increased steadily in all regions until 2 August, reaching the highest value of 0,425% in the ARS on this date. It is interesting to note that total N content, unlike the carbohydrate fractions dis= cussed previously, increased steadily throughout the autumn and winter to reach the season's high at the final analysis on 2 August. When the younger shoot regions were sampled for the first time, they had a higher total N concentration than the older regions at that time. This difference soon disappeared, and up to 1 June no shoot region had a definitely higher total N concentration that the rest. On 1 July and 2 August however, the BRP had somewhat lower total N values than most other regions.

With the exception of the ARS, initial total N concentration of fresh mass in the Salt Creek shoot regions was higher than the corresponding values in those of Jacquez. These values decreased to a minimum during February - March. During March, Salt Creek concentrations were very similar to those of Jacquez. From 1 April total N in all Salt Creek regions increased steadily until 2 August, attaining values similar to those of Jacquez. The ARS had the highest value on 2 August (0,419%). As in Jacquez, the younger shoot regions of Salt Creek at the first sampling had a higher total N concentration than those of the older regions at the same time.

The nitrogenous compounds in the shoots were initially derived from store reserves in the roots and to a lesser extent in the aboveground permanent wood of the vine. Later, absorption by the roots also contributed. As the mass of the shoots in=creased, total N concentration dropped because of a wider distribution of the available amount. The total N concentration did not start to increase until shoot growth had slowed down. This increase indicates the extent of N absorption by the roots at this stage.

The soluble N concentration of fresh mass in all regions of Jacquez followed similar patterns to total N, the only real difference being the dip in the values of the BRP and MRP and increases in the BRS and ARS during June. These changes were reversed during July. This can only be explained by retranslocation, the cause of which is unknown. Except for the high value for the base of the main shoot on 3 November, and those of the basal and apical secondary shoots on 1 July, the highest soluble N values were recorded on 2 August.

The graph of soluble N concentration of fresh mass for Salt Creek is more com= plicated, showing rapid decreases from initial high values to low levels on 2 February in all regions. All regions then showed increases to peak levels on 1 March, followed by decreases, to reach a second series of still lower values than the first on 1 April. After that there were increases in all regions, cul= minating in a second series of peak levels being reached in the BRP and MRP and BRS on 1 June. During June the BRP and MRP again gave an indication of the dip in concentration found in Jacquez at the same time. The general increases in soluble N concentration in all Salt Creek regions from 1 April to 2 August were much lower than those in Jacquez, resulting in the Salt Creek values for 2 August being about 50% of those recorded for Jacquez. The sudden increases in soluble N concentration in Salt Creek during February and the decreases during March are difficult to explain. There were no drastic temperature changes during that period, irrigation was adequate and no N fertiliser was applied at that stage. It seems as if there was a sudden growth flush which resulted in a temporary soluble N increase.

Kliewer (1967) determined the soluble and insoluble N concentrations (dry mass basis) in the "fruiting" canes of Thompson Seedless grapevines. The canes at the first two sampling dates in January and February were at the same stage of development as those of the shoots (canes) in this study in July and August. As in cane pruned vines the basal part of canes are pruned to "fruiting" canes, the results obtained by Kliewer can be compared with the concentrations in terms of dry mass in the BRP. The concentration on a dry mass basis obtained in the two studies on these dates are compared in Table 1. (The soluble and insoluble values recorded by Kliewer were added to obtain the total N). The total N concentration in the three compare very closely while soluble N increases in the order Salt Creek, Jacquez, Carignane.

4.3.2 Callusing, root and shoot growth

The following data on cuttings taken from different cane regions of Jacquez and Salt Creek vines on 2 August is presented in Table 2: Callus score, cutting mass, mass of shoots and roots formed on these cuttings, percentage dry mass, and sugar, starch, hemicellulose, total N, soluble N as concentration of dry mass. Correlation coefficients between callus score, root and shoot growth and the inde= pendent variables are also presented. These coefficients were determined from the replicate data. The most important results in this Table are as follows.

In Jacquez and Salt Creek, cutting mass decreased (though not always significant= ly) in the order BRP, MRP, ARP, BRS, MRS, ARS. This decrease gives an in= dication of the decrease in diameter of the cane from the base to the extremities.

In Jacquez there was no significant difference in callus score or root and shoot growth in cuttings taken from the different regions. Percentage dry mass differed significantly, cuttings from the MRS and ARS showing the highest percentage. Callus score, root growth and shoot growth showed no significant correlation with any variable. Two coefficients tended towards significance, namely shoot growth and cutting mass (positive) and shoot growth and percentage dry mass (negative).

Salt Creek presented a different picture. There was a significant difference in callus score depending on the region from which the cutting was taken. Callus score decreased in the order BRP, MRP, ARP, BRS, MRS, ARS and showed a significant positive correlation with cutting mass. This was the only significant correlation. Callus score also showed reasonably high positive coefficients with sugar and starch. As the actual differences in concentration of sugar and starch was relatively small, it is doubtful whether the concentration played a role in determining the callusing of the cuttings from different regions.

With percentage dry mass callus score also showed a negative correlation coeffi= cient which tended towards significance. From unpublished data (1978) it is known that thin cuttings from the extremities of Salt Creek canes callus better after leaching in water, while thick cuttings show no improvement. This lends sub= stance to the results shown here and it is very probable that drying out of the younger shoot regions affected the callusing ability of cuttings taken from them. However, the possibility of leaching of growth inhibitors from the thinner cuttings, with a resultant improvement of their callusing ability must also be borne in mind.

Root growth, very similarly to callusing, showed reasonably high correlation coefficients with cutting mass (positive) and percentage dry mass (negative). This suggests that root growth was affected by the same factors as callusing. Other reasonably high but not significant correlation coefficients in Salt Creek were negative ones between root and shoot growth and hemicellulose concentration. There was very little difference in hemicellulose concentration between cuttings from different regions, and the possibility that it could have had any influence on root and shoot growth must therefore be ruled out. Except for the relation= ship between cutting mass and callusing in Salt Creek which could just as well have been coincidental, there was no clear picture as to which factors affected callusing, root growth and shoot growth.

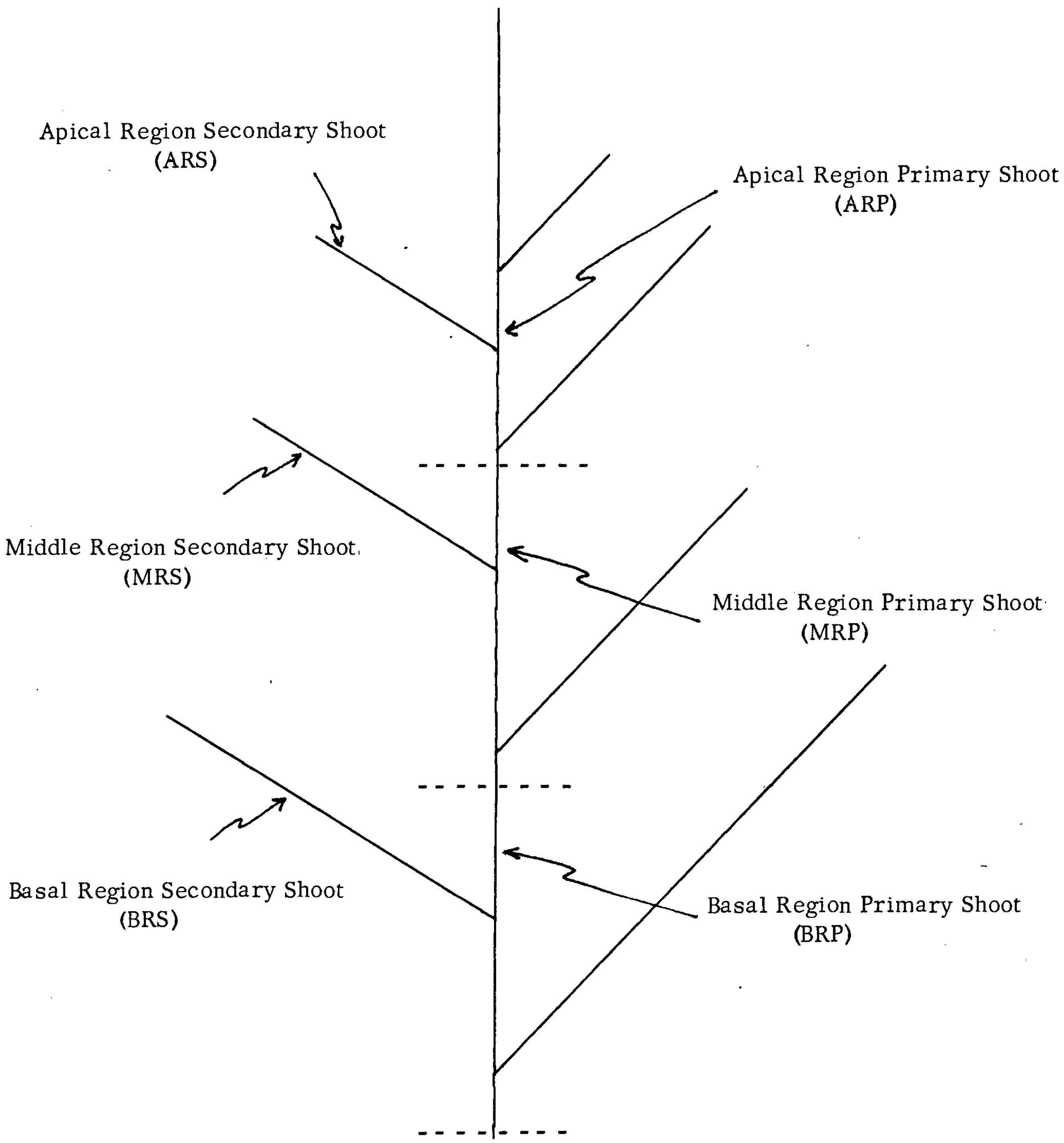


Fig. 4 - Different regions of primary shoot and position of secondary shoots

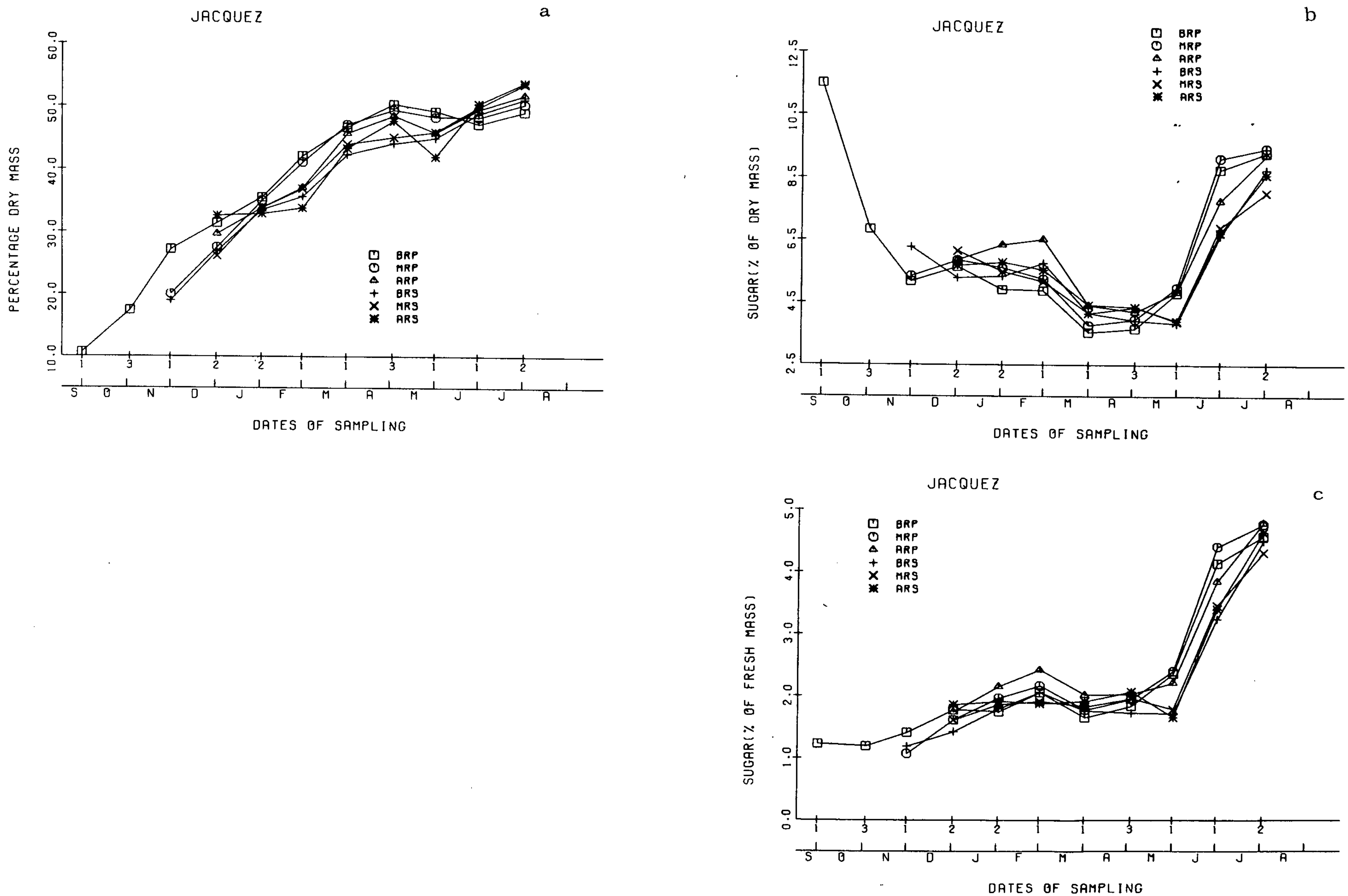


FIG. 5 - Seasonal changes in concentration of dry mass of different Jacquez shoot regions (a), sugar in these regions as concentration of dry mass (b) and fresh mass (c)

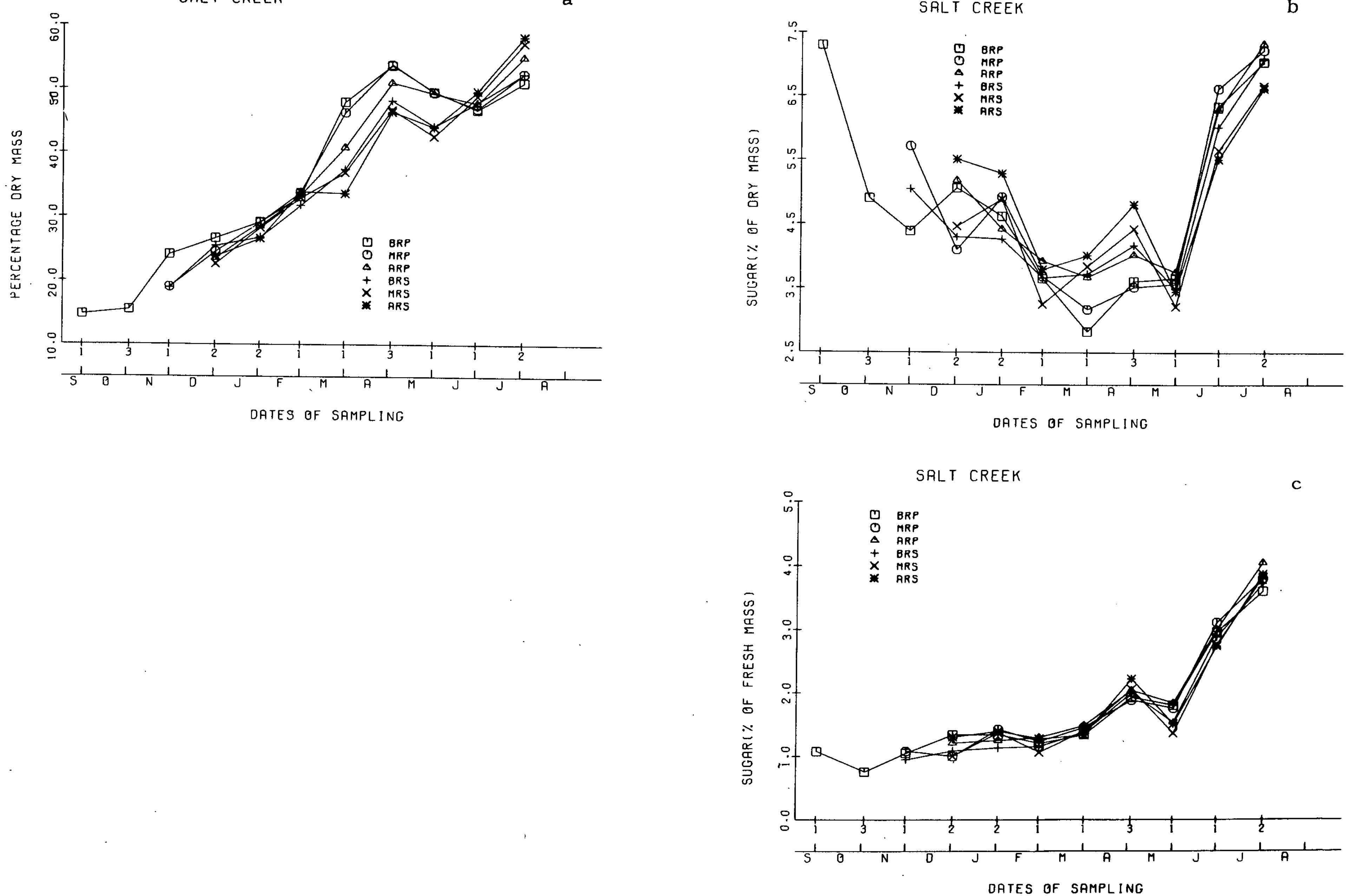


FIG. 6 - Seasonal changes in concentration of dry mass of different Salt Creek shoot regions (a), sugar in these regions as concentration of dry mass (b) and fresh mass (c)

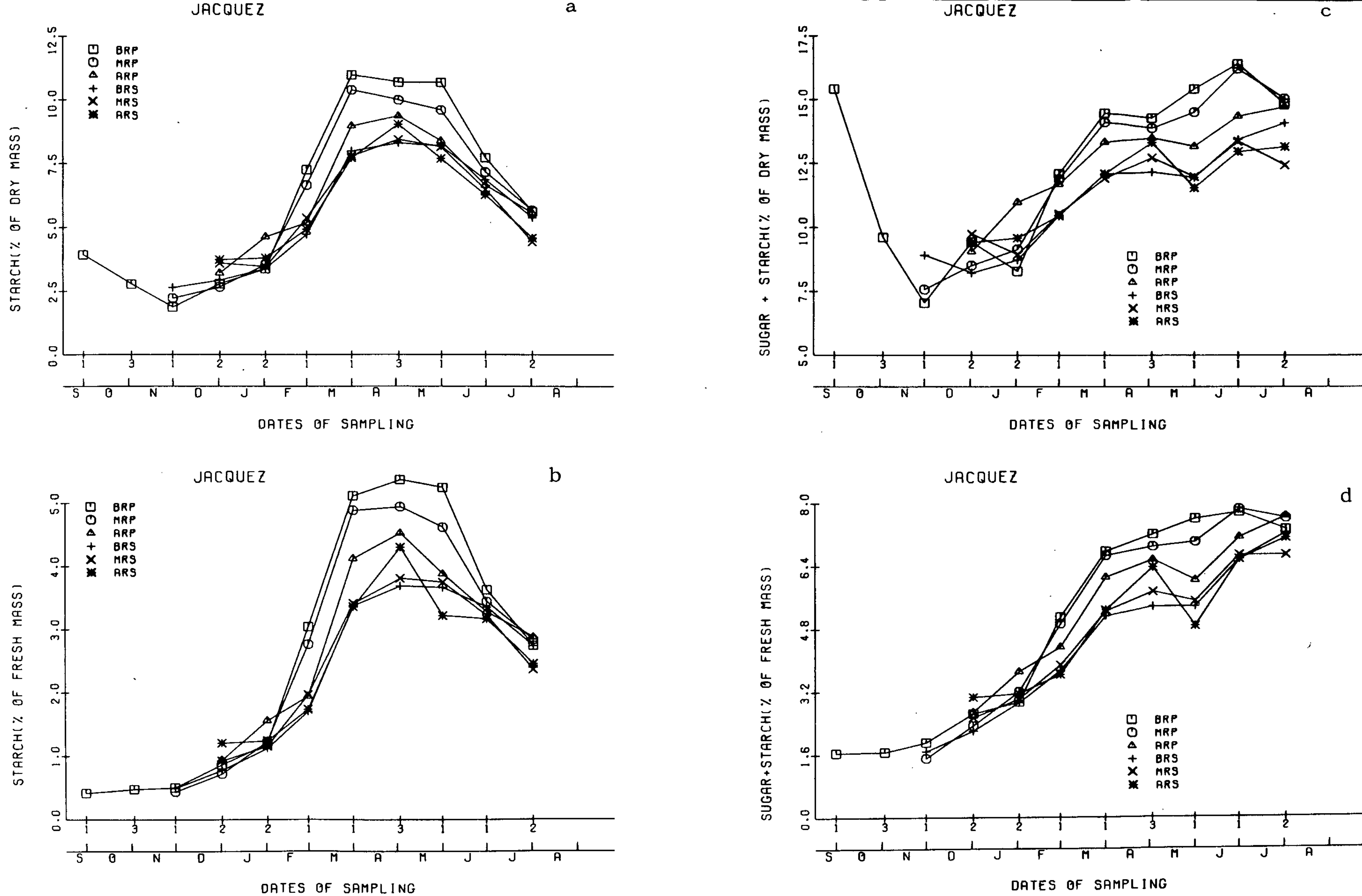


FIG. 7 - Seasonal changes in starch concentration of dry mass (a) and fresh mass (b), sugar + starch concentration of dry mass (c) and fresh mass (d), in different Jacquez shoot regions

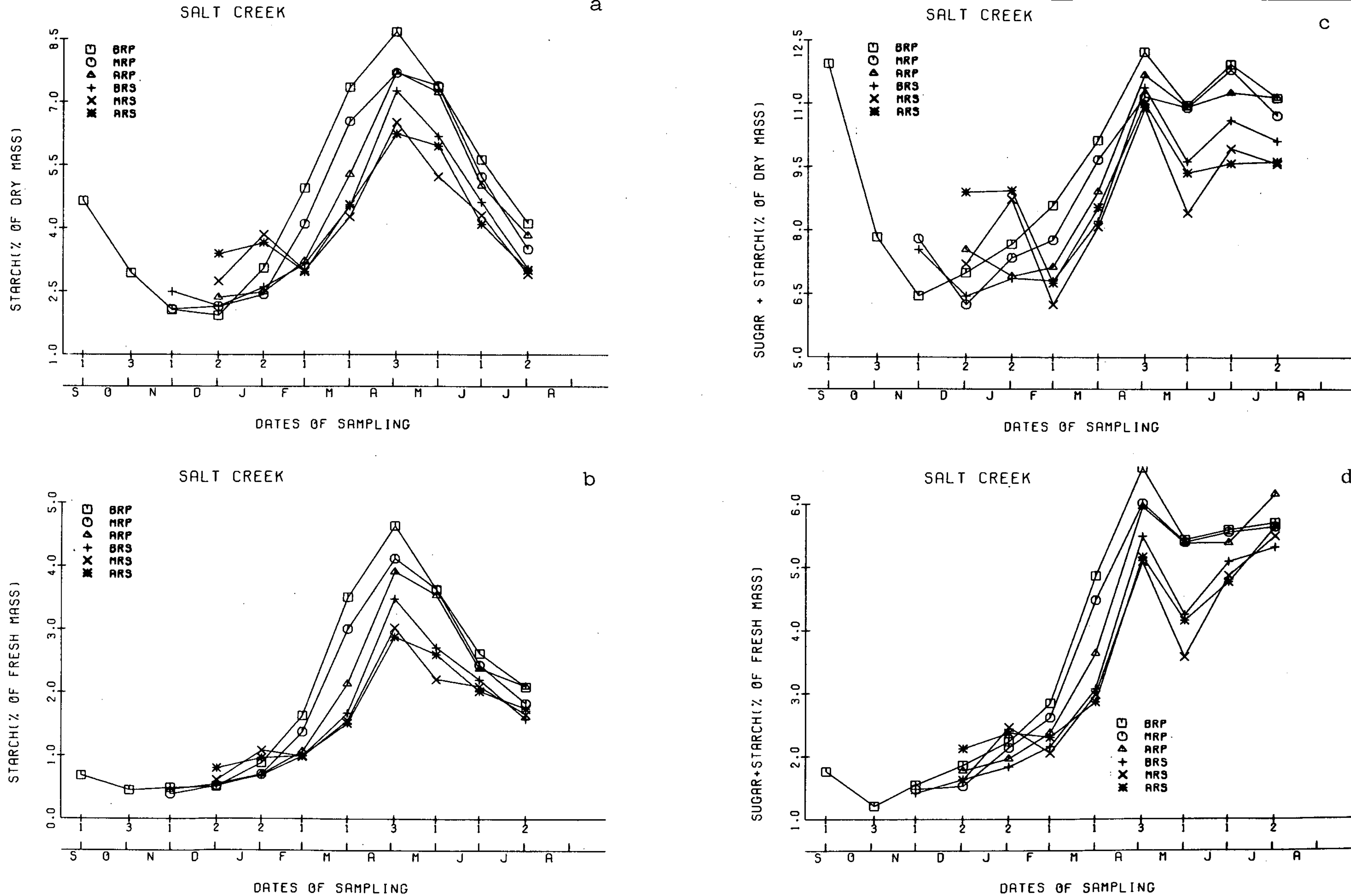


FIG. 8 - Seasonal changes in starch concentration of dry mass (a) and fresh mass (b), sugar + starch concentration of dry mass (c) and fresh mass (d), in different Salt Creek shoot regions

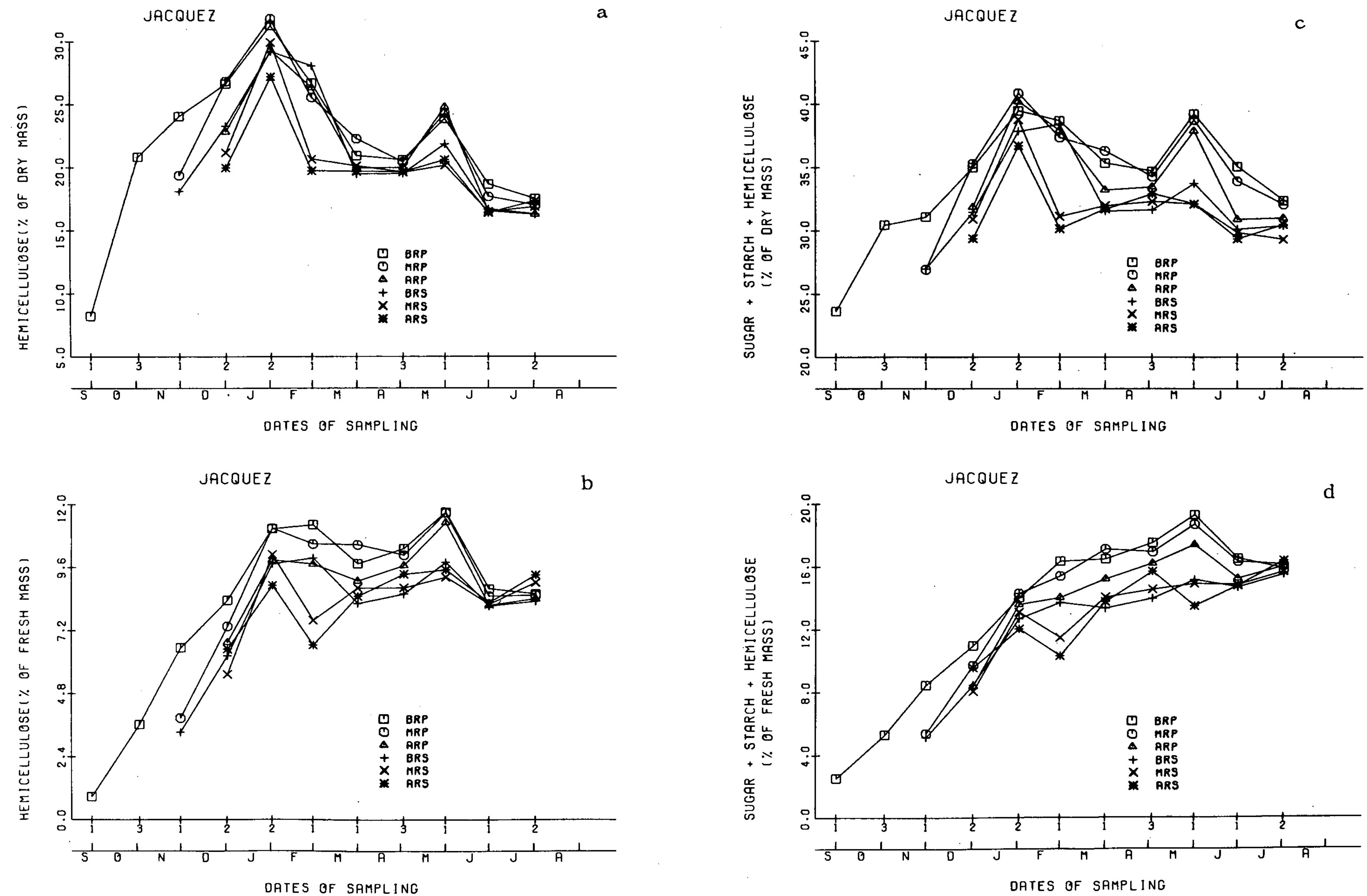


FIG. 9 - Seasonal changes in hemicellulose concentration of dry mass (a) and fresh mass (b), sugar + starch + hemicellulose concentration of dry mass (c) and fresh mass (d), in different Jacquez shoot regions

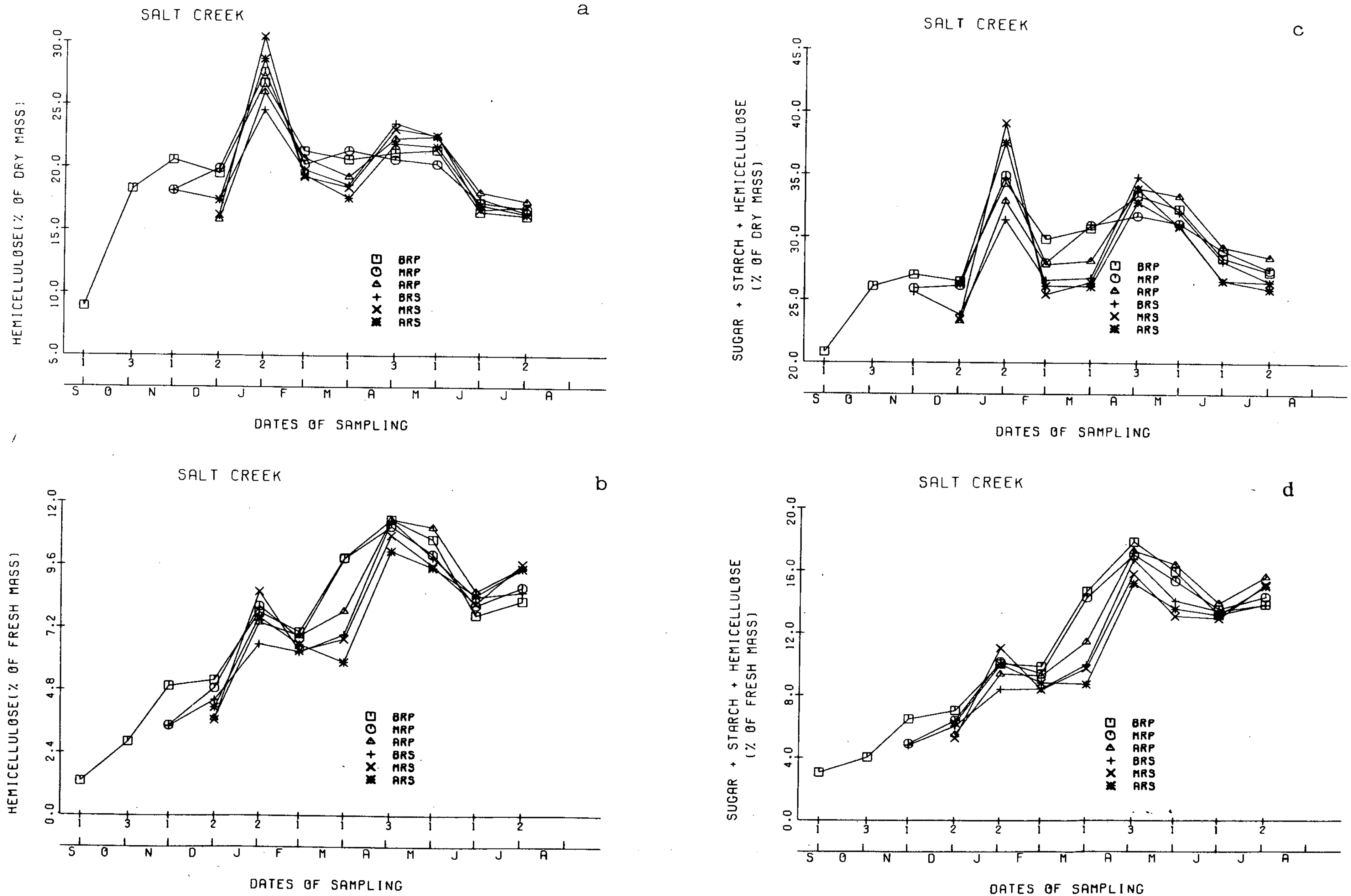


FIG. 10 - Seasonal changes in hemicellulose concentration of dry mass (a) and fresh mass (b), sugar + starch + hemicellulose concentration of dry mass (c) and fresh mass (d), in different Salt Creek shoot regions

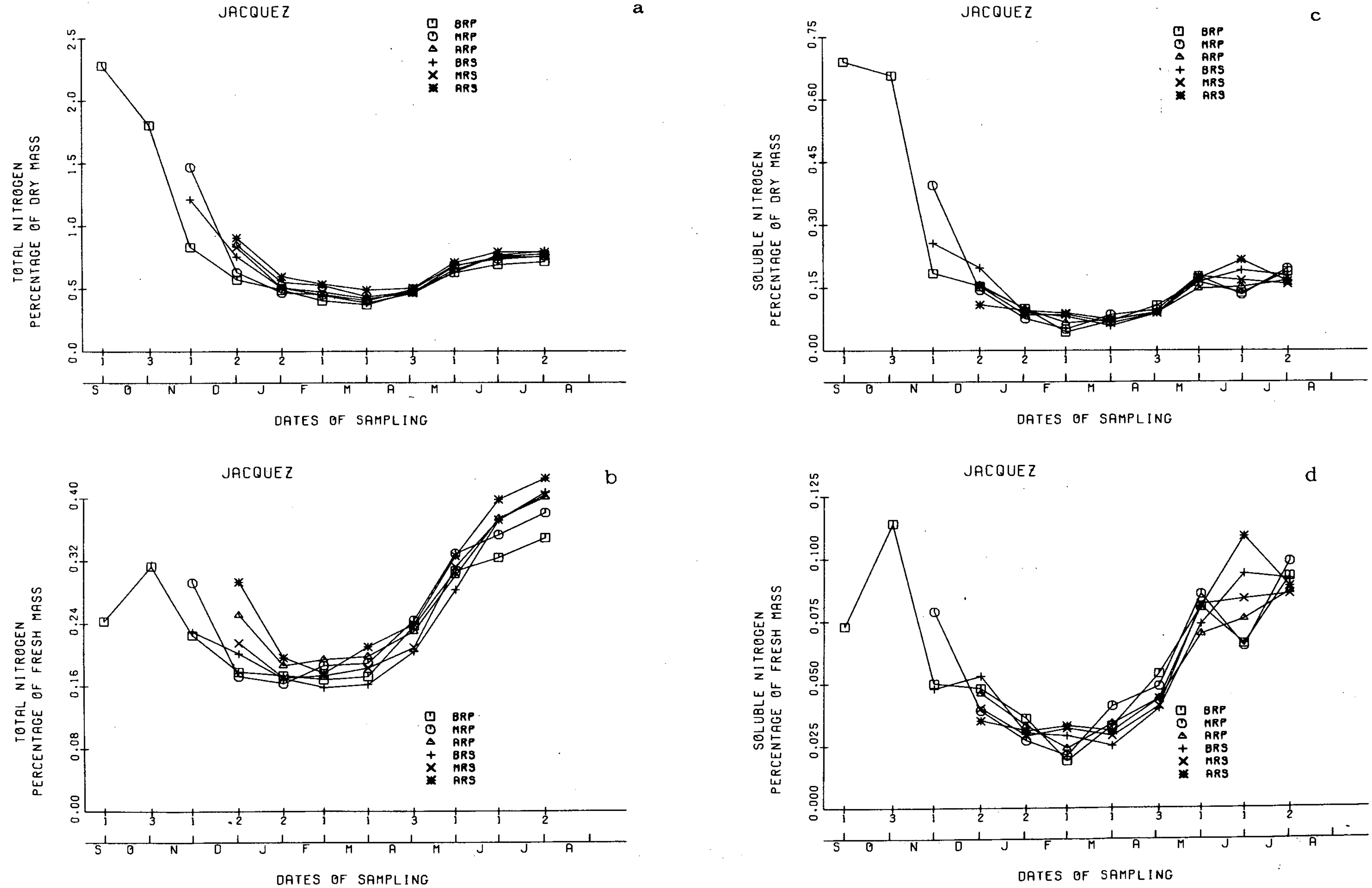


FIG. 11 - Seasonal changes in total N concentration of dry mass (a) and fresh mass (b), soluble N concentration of dry mass (c) and fresh mass (d), in different Jacquez shoot regions

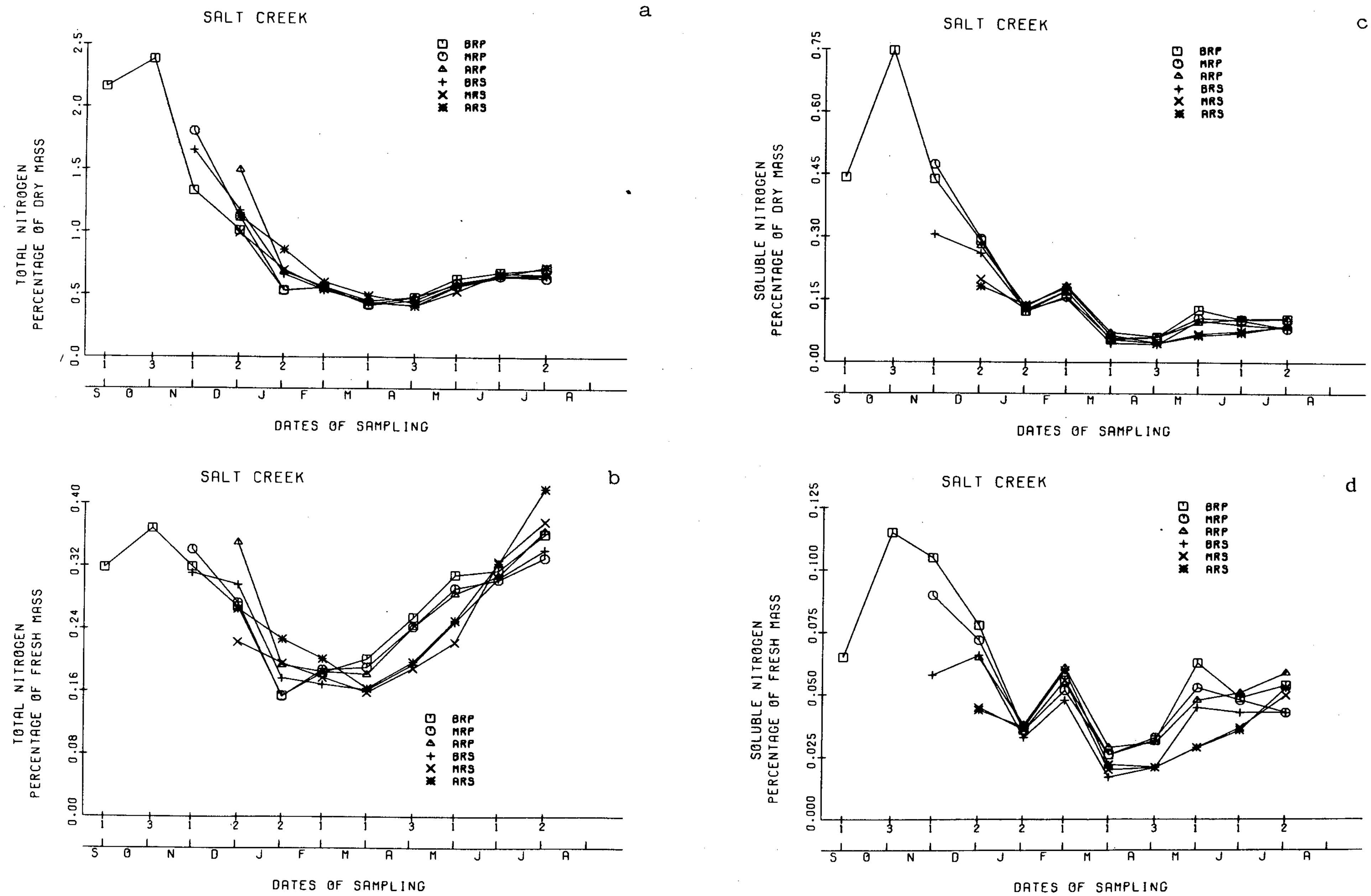


FIG. 12 - Seasonal changes in total N concentration of dry mass (a) and fresh mass (b), soluble N concentration of dry mass (c) and fresh mass (d), in different Salt Creek shoot regions

TABLE 1 - Comparison of the soluble and total N concentrations of dry mass recorded in the BRP of Jacquez and Salt Creek during July and August with those in Carignane canes (cane pruned) recorded by Kliewer (1967) in the seasonally corresponding months.

Month	Jacquez		Salt Creek		Month	Carignane	
	Soluble N	Total N	Soluble N	Total N		Soluble N	Total N
July	0, 14	0, 69	0, 10	0, 68	January	0, 23	0, 63
August	0, 19	0, 71	0, 11	0, 71	February	0, 24	0, 68

TABLE 2 - Effect of the position from which the cutting is taken on the mother vine of Jacquez and Salt Creek on the average mass of the cuttings, the score of callus formed after 18 days, the mass of roots and shoots formed during one year's growth of the cutting, as well as percentage dry mass of the cuttings and sugar, starch, hemicellulose, total nitrogen and soluble nitrogen as concentration of dry mass

Rootstock	Part of shoot from which cutting was taken	Callusing study		Rooting and shoot growth study			Percent dry mass	Sugar %	Starch %	Sugar + Starch %	Hemicellulose %	Sugar + Starch + Hemicellulose %	Total Nitrogen %	Soluble Nitrogen %
		Cutting mass(g)	Score	Cutting mass(g)	Root mass per cutting (g)	Shoot mass per cutting (g)								
Jacquez	BRP	e 28,44	a 24,8	c 29,17	a 25,53	a 7,38	a 49,13	c 9,34	b 5,59	d 14,93	a 17,50	c 32,43	a ,711	a ,188
	MRP	d 23,94	a 25,5	b 17,45	a 26,07	a 6,46	b 50,37	c 9,49	b 5,64	d 15,13	a 16,99	c 32,19	ab ,757	a ,196
	ARP	c 14,29	a 22,7	a 13,07	a 21,80	a 5,47	c 51,84	c 9,26	b 5,54	d 14,80	a 16,25	bc 31,05	b ,773	a ,167
	BRS	b 11,63	a 26,4	a 10,66	a 23,73	a 6,40	bc 51,21	bc 8,80	b 5,36	c 14,16	a 16,27	ab 30,43	b ,796	a ,179
	MRS	b 11,62	a 23,6	a 9,56	a 24,92	a 6,32	d 53,60	a 8,06	a 4,42	a 12,48	a 16,87	a 29,35	ab ,754	a ,159
	ARS	a 10,04	a 25,0	a 9,25	a 22,48	a 5,52	d 53,82	b 8,61	a 4,61	b 13,22	a 17,33	b 30,55	b ,797	a ,164
	Correlation coefficient for callus score	0,14	-	-	-	-	-0,33	0,13	0,19	0,16	0,15	0,22	0,21	0,57
	Correlation coefficient for root mass	-	-	0,55	-	-	-0,53	0,17	0,20	0,19	0,45	0,39	-0,67	0,65
	Correlation coefficient for shoot growth	-	-	0,78	-	-	-0,74	0,25	0,35	0,30	0,43	0,48	-0,21	0,64
Salt Creek	BRP	f 23,07	bc13,1	e 23,55	a 20,12	a 6,15	a 51,00	a 7,09	b 4,10	b 11,19	a 16,14	a 27,33	b ,709	a ,106
	MRP	e 17,71	c16,0	d 16,50	a 15,52	a 4,51	ab 52,41	a 7,28	ab 3,49	ab 10,77	a 16,72	a 27,03	a ,632	a ,082
	ARP	d 14,33	ab 9,5	c 13,24	a 14,46	a 4,98	bc 55,00	a 7,38	b 3,82	b 11,20	a 17,30	a 28,49	a ,664	a ,107
	BRS	c 9,88	ab 8,6	b 10,36	a 17,03	a 6,31	ab 52,36	a 7,13	a 3,03	ab 10,16	a 16,37	a 26,52	a ,653	a ,083
	MRS	b 7,56	a 6,8	b 9,84	a 14,28	a 4,94	c 57,20	a 6,71	a 2,90	a 9,61	a 16,87	a 26,48	a ,660	a ,088
	ARS	a 7,12	a 5,5	a 8,32	a 15,83	a 6,05	c 58,20	a 6,67	a 3,00	a 9,67	a 16,27	a 25,99	b ,720	a ,090
	Correlation coefficient for callus score	0,86	-	-	-	-	-0,79	0,69	0,66	0,73	-0,01	0,42	-0,18	0,05
	Correlation coefficient for root mass	-	-	0,72	-	-	-0,68	0,09	0,52	0,39	-0,78	-0,04	0,48	0,30
	Correlation coefficient for shoot growth	-	-	0,02	-	-	-0,15	-0,29	-0,05	-0,15	-0,77	-0,37	0,65	0,53

a - f : Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

r (5%) = 0,811

r (1%) = 0,917

SECTION 5

EFFECT OF CUTTING DATE AND COLD STORAGE OF CUTTINGS TAKEN AT DIFFERENT TIMES DURING THE DORMANT PERIOD ON THE DRY MASS, CARBOHYDRATE LEVELS AND DEGREE OF CALLUSING

5.1 Introduction

Changes in the composition of the cane during the dormant period were reported in section 4. The question arises as to whether collection of cuttings at the time of higher carbohydrate levels very early during the dormant period (be= ginning of May) could positively affect callusing, and if so, whether this advantage could be exploited for grafting in August. This would necessarily require storage of cuttings from that early date until August.

It is known (Bosman, 1976) that a procedure of very early cutting, storage under cool conditions in sand, and soaking of the cuttings in water after cutting and before grafting does improve the callusing ability. The best conservation of carbohydrates would be obtained if the cuttings were stored at a low temperature.

This study was undertaken to investigate two factors. Firstly the sugar, starch and hemicellulose concentration and callusing ability of cuttings taken at intervals during the dormant period. Secondly, possible changes in these carbohydrates and callusing ability during cold storage at $0^{o}C$ from the earlier cutting dates until 1 August.

5.2 Procedure

The experiment was laid out as a factorial arrangement of treatments. There were two factors, namely cultivar (Jacquez and Salt Creek) and cutting date with or without cold storage as follows: 2 May, 1 June, 1 July, 1 August, 1 September for immediate sampling and 2 May, 1 June, 1 July for cold storage until 1 August. Each factor combination was replicated 6 times in a completely randomized layout. Each replicate had 20 cuttings for analysis and the same number for callusing. All cuttings were of the diameter used for callusing.

Cold storage of cuttings was carried out in plastic bags at $0^{o}C$. The fresh mass of these cuttings were determined before and after cold storage. They were dip= ped in tap water for 10 minutes once per month while being stored. Callusing and scoring of callus after 21 days was done according to the procedure described in section 3.4. After recording of the fresh and dry mass, samples were analysed for sugar, starch and hemicellulose. The analytical results were expressed as percentage of dry mass for those sampled immediately, and as percentage of initial dry mass for those which were cold stored.

5.3 Results and discussion

The cuttings used in this study would more or less correspond with those taken from the BRP, MRP, ARP and BRS in section 4. The effect of cold storage after different cutting times on the percentage change in fresh mass of Jacquez and Salt Creek cuttings is presented in Table 3.1.

The changes were relatively small and except for Salt Creek cut on 2 May which lost some fresh mass, there were gains, indicating an uptake of water during cold storage. There was therefore no possibility that drying out of the cuttings could have affected callusing.

The effect of time of cutting and cold storage on the percentage dry mass of Jacquez and Salt Creek cuttings is presented in Table 3.2. The effect of the same factors on the sugar, starch and hemicellulose as concentration of dry mass or initial dry mass is shown in Table 3.3.

The percentage dry mass in both cultivars changed very little from 2 May to 1 August. Jacquez showed a slightly higher value on 2 May, whereas Salt Creek reached a peak on 1 June (compared to 3 May in section 4.3.1.1). On 1 September both cultivars had a lower percentage dry mass than at any other time. This can= not be explained by a loss of sugar, starch and hemicellulose, and was probably due to an increase of water in the canes. All the cold stored cuttings showed decreases in percentage dry mass. These decreases probably had two causes. Firstly, and most important, a loss of carbohydrates which occurred in all cases. Secondly, and less important, increases in the fresh mass of the cuttings, which have already been discussed.

The loss in sugar + starch + hemicellulose during August was larger in Salt Creek than in Jacquez. This indicates that the Salt Creek was already more physiological= ly active during August than Jacquez. The cold storage conditions caused statis= tically significant starch to sugar conversion in the cuttings taken in May and June in Jacquez. The concentration of sugar + starch, and their combined concentra= tions with hemicellulose, decreased in all cuttings during cold storage, the losses being greater for the longer periods of storage and more pronounced in Jacquez than in Salt Creek. There was thus no advantage, as far as carbohydrate content is concerned, in taking cuttings early and cold storing them.

The effect of time of cutting and cold storage on the callusing of Jacquez and Salt Creek cuttings after 21 days is presented in Table 3.4.

The most striking results was the loss of callusing ability at the apex between 2 May and 1 June. After this the callusing ability of the apex increased slowly in Jacquez, and within one month in Salt Creek. Callusing at the base of Jacquez also decreased from 2 May to 1 June, but not as dramatically as at the apex. The Salt Creek base only changed slightly as far as callusing ability was concerned between these two dates.

Comparison of the results in Tables 3.3 and 3.4 shows that the large differences in callusing at the apex of the cuttings could not have been caused by differences in sugar, starch and hemicellulose concentrations. The highest level of sugar + starch in Salt Creek on 1 June coincided with the poorest callusing results at the apex. There was therefore a more important factor or factors than carbohydrate concentration which influenced callusing at this stage.

Cold storage of cuttings generally depressed callusing, and the longer the period, the greater was the effect. Only at the base of the Salt Creek cuttings taken on 1 July a slight improvement in callusing after cold storage was recorded. These results therefore suggest that any possible advantage that may be obtained by early cutting cannot be conserved by cold storage at $0^{o}C$.

These results appear to be in contrast with those of Bosman (1976). However, there were two important differences between the procedure followed in this study and the method used by Bosman. Firstly, the average temperature in storage under sand would be well above $0^{o}C$. Secondly, Bosman had twice soaked the cuttings in water. It seems as though the success obtained by Bosman was caused by factors other than higher carbohydrate content during early cutting.

TABLE 3.1 - Effect of cold storage after different cutting times on the change in fresh mass of Jacquez and Salt Creek cuttings

Rootstock	% change in fresh mass during cold storage		
	2 May	1 June	1 July
Jacquez	+ 3, 39	+ 2, 72	+ 2, 51
Salt Creek	- 1, 77	+ 0, 96	+ 0, 78

TABLE 3.2 - Effect of time of cutting and cold storage on the percentage dry mass of Jacquez and Salt Creek cuttings

Rootstock	Dry mass (%)							
	Immediate sampling					Cold stored until 1 August		
	2 May	1 June	1 July	1 August	1 September	2 May	1 June	1 July
Jacquez	50, 0 D	49, 0 C	48, 9 C	49, 0 C	45, 7 A	45, 7 A	46, 6 A	46, 8 B
Salt Creek	51, 7 BCD	54, 2 C	52, 5 DE	52, 3 CDE	49, 3 A	50, 9 B	53, 2 E	51, 4 BC

A - E - Compare in the same horizontal row. Means accompanied by a common letter are not different at the 5% level

TABLE 3.3 - Effect of the time of cutting and cold storage on the sugar, starch and hemicellulose of Jacquez and Salt Creek cuttings. Data for immediate sampling is given as concentration of dry mass while data for the cold stored cuttings is given as concentration of initial dry mass

Component	Rootstock	Concentration of components							
		Immediate sampling (% of dry mass)					Cold stored until 1 August (% of initial dry mass)		
		2 May	1 June	1 July	1 August	1 September	2 May	1 June	1 July
Sugar	Jacquez	3,07 A	3,93 A	6,83 E	9,51 F	7,23 E	6,45 DE	5,42 C	5,77 CD
	Salt Creek	3,17 A	4,08 ABC	4,98 C	5,80 D	3,55 AB	3,42 AB	4,20 ABC	4,39 BC
Starch	Jacquez	10,74 D	10,51 D	8,97 C	7,10 B	8,51 BC	5,01 A	8,50 BC	8,13 BC
	Salt Creek	7,96 C	7,30 BC	5,70 AB	5,16 A	6,28 ABC	6,97 ABC	6,94 ABC	5,89 AB
Sugar + starch	Jacquez	13,81 B	14,44 B	15,80 BC	16,61 C	15,74 BC	11,46 A	13,92 B	13,90 B
	Salt Creek	11,13 A	11,38 A	10,68 A	10,96 A	9,83 A	10,39 A	11,14 A	10,28 A
Hemicellulose	Jacquez	20,46 B	18,43 A	18,32 A	17,19 A	16,76 A	16,35 A	16,75 A	18,19 A
	Salt Creek	20,68 B	18,03 A	18,79 A	18,51 A	17,12 A	17,96 A	17,71 A	18,15 A
Sugar + starch + hemicellulose	Jacquez	34,27 C	32,87 BC	34,12 C	33,80 BC	32,50 BC	27,81 A	30,67 B	32,09 BC
	Salt Creek	31,81 B	29,41 AB	29,47 AB	29,47 AB	26,95 A	28,35 AB	28,85 AB	28,43 AB

TABLE 3.4 - Effect of the time of cutting and cold storage on the callusing of Jacquez and Salt Creek cuttings after 21 days.

Component	Rootstock	Callus score							
		Immediate callusing					Cold stored until 1 August		
		2 May	1 June	1 July	1 August	1 September	2 May	1 June	1 July
Apex	Jacquez	27,8 E	3,9 B	2,7 AB	12,5 C	16,0 D	0,0 A	0,5 A	0,7 A
	Salt Creek	17,5 CD	0,3 A	18,7 D	11,3 B	15,1 C	0,0 A	1,0 A	2,0 A
Base	Jacquez	22,4 H	11,0 C	14,3 E	17,0 F	19,5 G	4,7 A	6,9 B	13,2 D
	Salt Creek	19,2 E	18,9 D	19,4 F	14,8 B	20,8 G	9,0 A	16,4 C	22,2 H

A - H : Compare in the same horizontal row. Means accompanied by a common letter are not different at the 5% level

SECTION 6

EFFECT OF CALLUSING PERIOD AND DEGREE OF CALLUSING ON THE LEVELS OF CARBOHYDRATE AND NITROGEN FRACTIONS AND OTHER MACRO-ELEMENTS IN DIFFERENT REGIONS OF CUTTINGS AND IN SCIONS

6.1 Introduction

The cutting or scion used for grafting is dependent on its reserves of carbohydrates, nitrogenous substances and other elements for the development of callus and the initial development of roots and shoots. The first development to take place under suitable conditions is the formation of callus tissue at the base and apex of the cutting. The question arises as to whether the reserve components necessary for this are drawn from the immediate vicinity of these areas, whether there is translocation from another part of the cutting towards it and whether there is ac= cumulation of these components at the areas of callusing. This study was under= taken to investigate these aspects.

6.2 Procedure

Cuttings for callusing of Jacquez and Salt Creek and scions of Waltham Cross were collected at random at the beginning of August 1976 and held at $0^{o}C$ until the start of the experiment on 2 September 1976.

Due to limitations with regard to the quantity of cuttings available and callusing facilities, a formal statistical design could not be followed. The statistical ana= lysis was done according to advice of a biometrican. The experimental layout was as follows. For each cultivar there was an initial control sampling on 2 September and three callusing periods from that date until 9, 16 and 23 September respectively. Three replicates of 40 cuttings or scions were used for each period. The initial fresh mass of each replicate was recorded. For practical reasons, the 40 cuttings per replicate were put into two callusing bags which were placed next to each other in the callusing room. Bags were arranged in the callusing room to a randomized block design. Cuttings or scions taken out after seven days were scored as a whole for each replicate, while those taken out after 14 and 21 days were first divided into two groups, namely those showing good and poor callus formation at the apex of the rootstock cuttings and the base of the scions, after which the callus was scored.

For purpose of analysis, rootstock cuttings of all the sampling dates were divided into four regions as shown in Fig. 13. The apical regions did not contain a node while the basal region always contained one node with a bud.

The final samples were analysed for sugar, starch, hemicellulose, total N, soluble N, P, K, Ca and Mg. Analytical results were expressed as concentration of initial dry mass for the different cutting regions, scions and callusing groups, as well as total content per cutting or scion. Content values were corrected to the average initial cutting or scion mass for each cultivar. Values for 16 and 23 September were calculated separately for good and poor callusing, but they were also combined in a weighted mean which was comparable with those of 2 and 9 September.

6.3 Results and discussion

6.3.1 Callusing

The effect of the callusing period on callus formation on Jacquez and Salt Creek cuttings and Waltham Cross scions is presented in Table 4.

Waltham Cross was the first to start callusing, followed by Salt Creek and lastly Jacquez. After the three cultivars had callused for periods of 14 and 21 days, Jacquez was found to have developed the most callus tissue at the apex and the least at the base, with the apex callusing slightly better than the base. Salt Creek and Waltham Cross callused equally poorly at the apex and equally well at the base. Being the scion, good callusing at the base is an advantage for Waltham Cross. The poor callusing at the apex is a disadvantage to Salt Creek as a rootstock cutting; this is probably the main reason for the poor percentage take which is often obtained in grafting on Salt Creek. On the whole, callusing activity was directed more to= wards the base in Salt Creek and more towards the apex in Jacquez.

6.3.2 Sugar, starch and hemicellulose

The effect of the callusing period on sugar, starch and hemicellulose content in Jacquez and Salt Creek cuttings and Waltham Cross scions is presented in Figs. 14a, b; 15a, b; 16a, b and Appendix 9.1. Appendix 9.1 also shows the change over the full period of 21 days in mg, and as a percentage of the initial value.

In all three cultivars, sugar showed the largest loss after the first 7 days, followed by smaller losses in Jacquez and Salt Creek after 14 and 21 days. Compared with this, Waltham Cross showed an increase after 21 days.

Starch in Waltham Cross and Jacquez increased during the first 7 and 14 days re= spectively, before decreasing again up to 21 days. These increases were probably the result of a sugar to starch conversion, the opposite of the change during early winter (section 4.3.1.2). This was probably caused by the warmer conditions to which the cuttings and scions were subjected during callusing. Salt Creek did not show the same initial starch increase, but the level remained fairly constant during the first week. Compared with the other two cultivars where the initial sugar content was much higher than that of the starch, the initial sugar content in Salt Creek was only slightly higher than that of the starch. The reason for the sugar to starch con= version apparently being absent in Salt Creek was most probably due to the fact that it had already partly taken place before the callusing period had commenced. More evidence for this deduction is given in later experiments.

Hemicellulose showed an initial increase in all three cultivars. This again is the opposite of the change that took place during June (section 4.3.1.2). It was pro= bably due to the conversion of sugar to hemicellulose which may have resulted from the higher temperature during callusing. All three cultivars showed a loss of hemicellulose after the initial increase, but in Salt Creek it increased a second time (which was not statistically significant) during the third week. This increase was also evident in later experiments, which might suggest the formation of new cell walls at this early stage.

With the combination of sugar, starch and hemicellulose content, Jacquez showed the lowest loss of carbohydrates (10,4%) over the three week period, while larger percentages were lost in Salt Creek (21,1%) and Waltham Cross (18,1%). These losses expressed in mg for Jacquez, Salt Creek and Waltham Cross were 154,4; 288,2 and 71,7 respectively. For the same cutting length, Salt Creek utilised a considerably larger amount of carbohydrates than Jacquez.

Four factors could have contributed to the losses in sugar + starch + hemicellulose. Firstly, the use of sugars for respiration. This was probably the most important factor. Energy was required for the normal physiological processes of the tissue, which were speeded up under the higher temperature, and for the synthesis of new components. Secondly, synthesis of components other than sugar, starch and hemicellulose, for instance cellulose, which is an important constituent of cell walls. Thirdly, a leaching of the sugars by water drops which had condensed on the inside surface at the top of the callusing bag, and dripped down on the cuttings. As the condensation was very slow this would have been a minor contributing factor. Fourthly, a loss because of the loss of small pieces of bark during the washing process before freeze-drying. Salt Creek and Waltham Cross showed an increased rate of utilisation of sugar + starch + hemicellulose during the second and third week. Compared to this, Jacquez showed an increased rate of utilisation during the the third week. This suggests that Jacquez was slower in resumption of ac= tivity.

The effect of the callusing period as well as the degree of callusing after 14 and 21 days on sugar and starch concentration of initial dry mass in four regions of Jacquez and Salt Creek cuttings and Waltham Cross scions, is presented in Figs. 17, 18, 19a and Appendices 10 - 15, 32.1. The effect of the same factors on hemicellu= lose and sugar + starch + hemicellulose concentration of initial dry mass is pre= sented in Figs. 20, 21, 19b and Appendices 16 - 19, 32.1.

The patterns of the changes in sugar, starch and hemicellulose were basically the same in all four cutting regions of Jacquez and Salt Creek. In Jacquez the AR showed a tendency towards lower sugar and starch concentration than the other regions after 7 to 21 days. The Salt Creek AR had a significantly lower starch content than the other regions after 21 days, and a tendency towards a lower sugar + starch content after 7 to 21 days. Apart from these differences, sugar, starch and hemicellulose where depleted or formed to the same extent in all regions. There were a few differences between the good and poor callusing groups, some of which were statistically significant. The most important of these were the following.

After 14 days, all four regions of the poor callusing Jacquez cuttings had a signi= ficantly higher sugar concentration than those which had callused well. This suggests that whereas the sugar was available, it was not being utilised. In Salt Creek there was a tendency for the poor callusing group to have a lower starch concentration. The concentration in the poor callusing group was significantly lower after 14 days in the SBR and after 21 days in the AR and SAR. It can be concluded that poor callusing at the apex of Salt Creek cuttings was connected with starch concentration, but because of the small differences in concentration between the good and poor callusing groups was not the sole cause of the callusing differences.

6.3.3 Total N and soluble N

The effect of the callusing period on total N and soluble N content in Jacquez and Salt Creek cuttings and Waltham Cross scions is presented in Figs. 14c, 15c, 16c and Appendix 9.1.

The small increase of total N in Jacquez and the small decrease in Salt Creek over the 21 day period was nog significant. Waltham Cross showed a significant loss after 21 days. However, all the changes were small and can probably be ascribed to experimental error, leaching of soluble N, and a loss of pieces of bark during the washing process. In all three cultivars soluble N increased significantly over the 21 day period. This was probably due to protein hydrolysis through which soluble N compounds were formed. The soluble N compounds were then available for resynthesis to protein in regions where protein was required. The increases in soluble N content at any stage must not be regarded as an index of the protein hydrolysis which had already taken place, as protein synthesis is undoubtably also utilising soluble N at the same time as hydrolysis is taking place. The soluble N content therefore only represents the labile pool of N compounds which are available for resynthesis at that stage.

The effect of the callusing period as well as the degree of callusing after 14 and 21 days on total N and soluble N concentration of initial dry mass in four regions of Jacquez and Salt Creek cuttings and Waltham Cross scions is presented in Figs. 22, 23, 19d and Appendices 20 - 23, 32.1.

Both Jacquez and Salt Creek showed a significant increase in total N concentration in the BR of the cuttings after 14 and 21 days. As these higher values cannot be explained by higher soluble N concentration alone, such increases must have been the result of translocation of soluble N to these regions, followed by conversion to insoluble N. In both cultivars there was also a tendency for the AR to have lower values after 14 to 21 days, although this was only significant in some cases. Soluble N concentration did not differ materially between the regions, the exception being Salt Creek which had significantly higher values in the BR after 14 and 21 days.

Few significant differences in total and soluble N between the good and poor callusing groups were found and these differences were small.

6.3.4 Phosphorus, potassium, calcium and magnesium

The effect of the callusing period on P, K, Ca and Mg content in Jacquez and Salt Creek cuttings and Waltham Cross scions is presented in Figs. 14d, 15d, 16d and Appendix 9.2.

With the exception of Mg in Salt Creek these elements in all three cultivars de= creased over the 21 day period. The percentage loss varied between 2,3 and 17,0 There were two possible causes for this loss. Firstly, leaching from the cutting or scion and secondly, a loss of small pieces of bark during the washing process before freeze - drying.

The effect of the callusing period as well as the degree of callusing after 14 and 21 days on the P, K, Ca and Mg concentration of initial dry mass in four regions of Jacquez and Salt Creek cuttings and Waltham Cross scions, is presented in Figs. 24, 25, 19d and Appendices 24 - 31, 32.2.

Jacquez showed a significantly lower K and Ca concentration in the AR of the initial sample. These lower concentrations in the AR can possibly be attributed to a difference in composition of the node and internode. After 7 days' callusing, these differences had disappeared, and it can be concluded that there must have been some translocation to bring this about.

The BR of Salt Creek had a significantly higher P concentration after 21 days, but apart from this there were no differences which could indicate translocation during the callusing period.

Only one noteworthy difference was found between the good and poor callusing groups: The significantly higher P concentration in the AR and SAR of the Salt Creek cuttings which had callused well after 21 days. These differences were probably the re= sult of the differences in callus formation and not the cause of it.

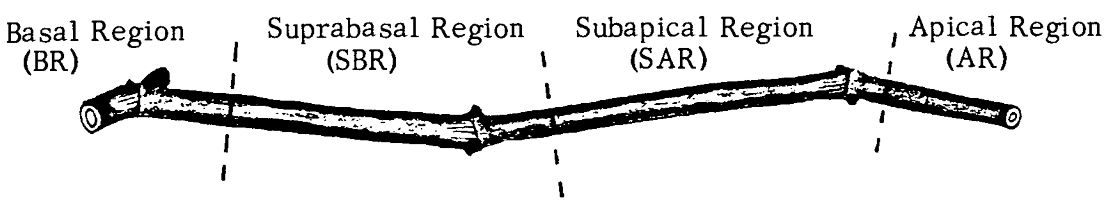


FIG. 13 - Division of cuttings into four regions for sampling

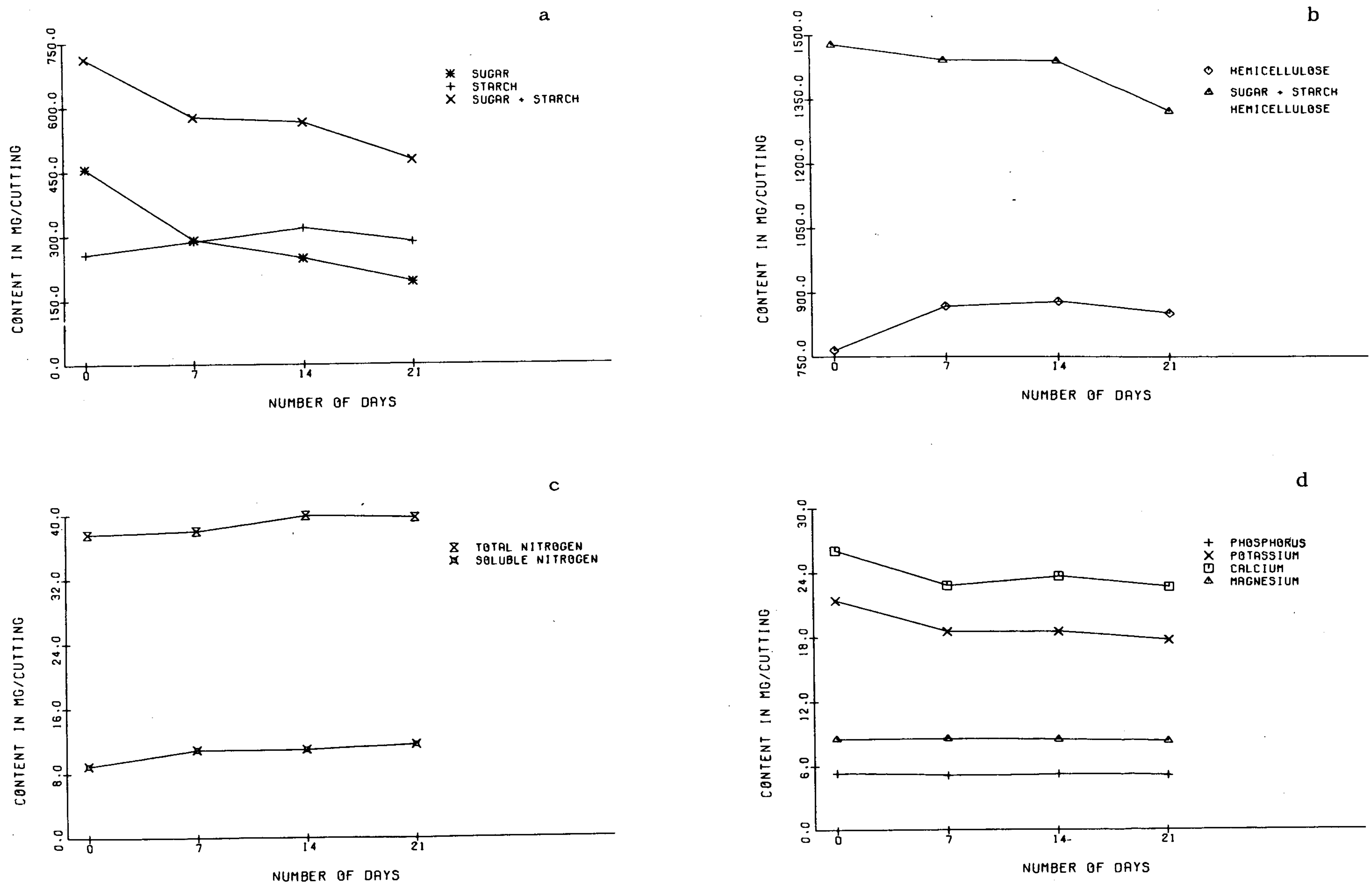


FIG. 14 - Effect of callusing period on sugar and starch (a), hemicellulose and sugar + starch + hemicellulose (b), total N and soluble N (c), phosphorus, potassium, calcium and magnesium (d), content in Jacquez cuttings

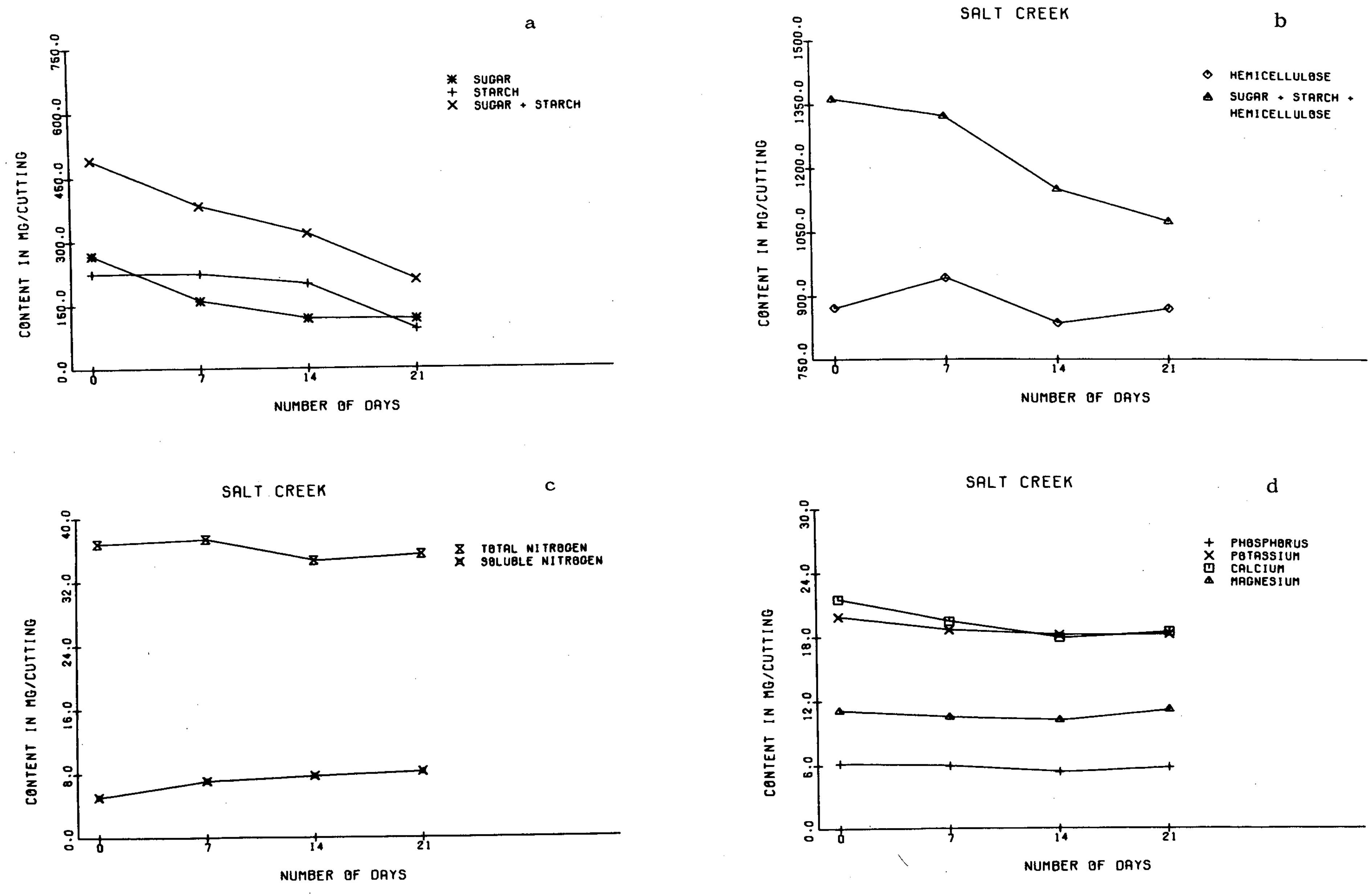


FIG. 15 - Effect of callusing period on sugar and starch (a), hemicellulose and sugar + starch + hemicellulose (b), total N and soluble N (c), phosphorus, potassium, calcium and magnesium (d), content in Salt Creek cuttings

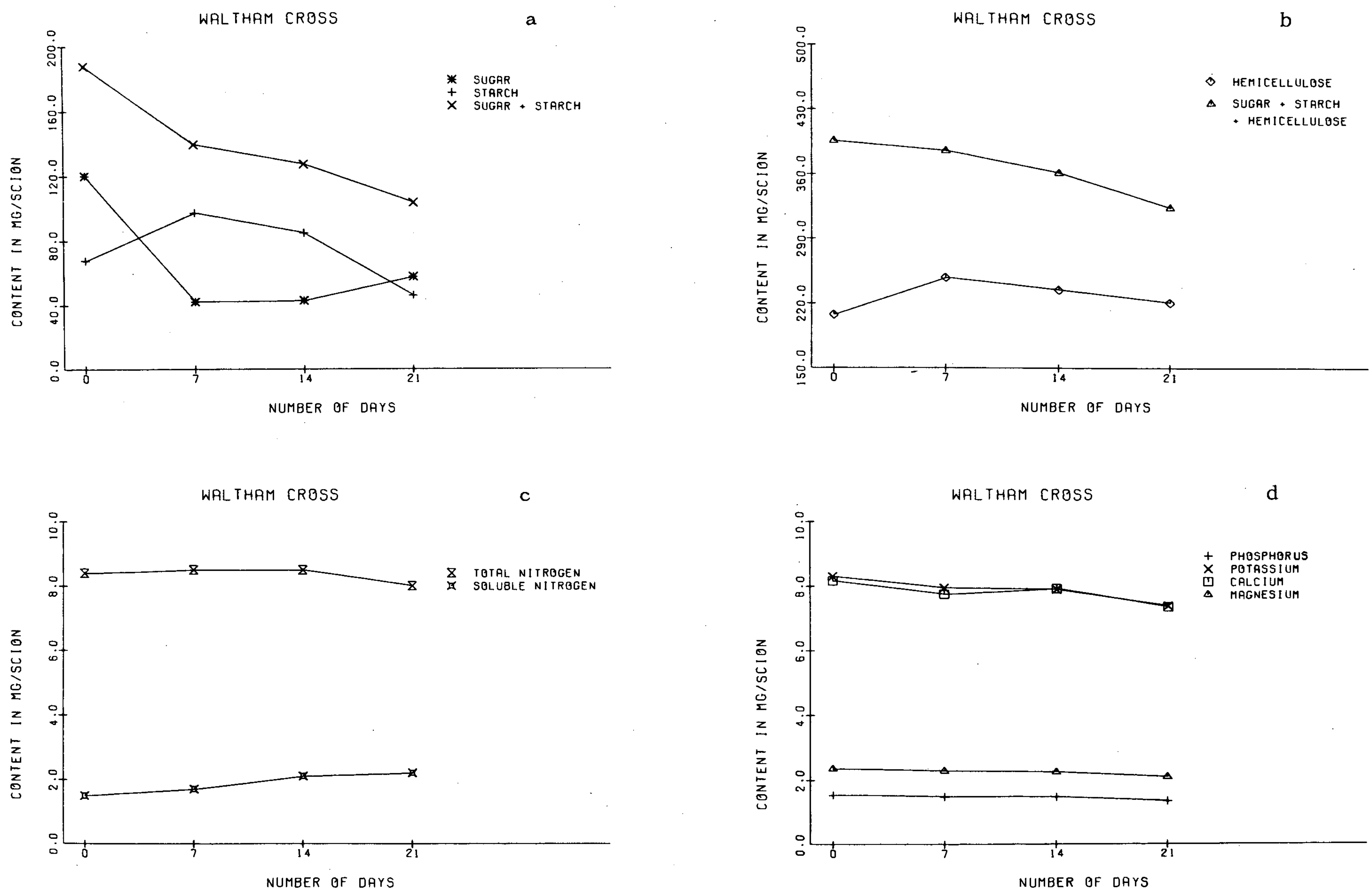


FIG. 16 - Effect of callusing period on sugar and starch (a), hemicellulose and sugar + starch + hemicellulose (b), total N and soluble N (c), phosphorus, potassium, calcium and magnesium (d), content in Waltham Cross scions

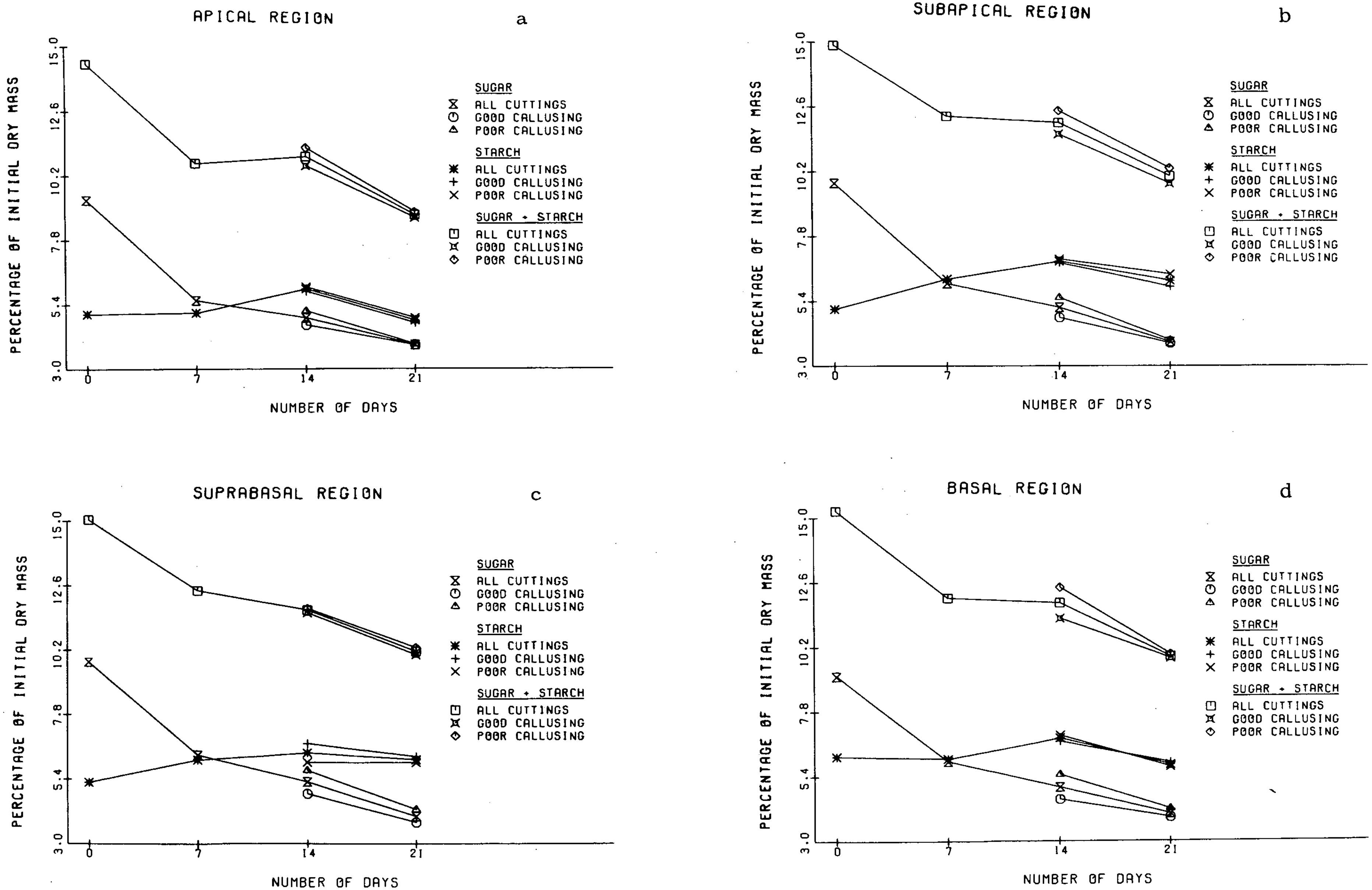


FIG. 17 - Effect of callusing period on sugar and starch concentration of initial dry mass in the apical (a), subapical (b), suprabasal (c), and basal (d), regions of Jacquez cuttings

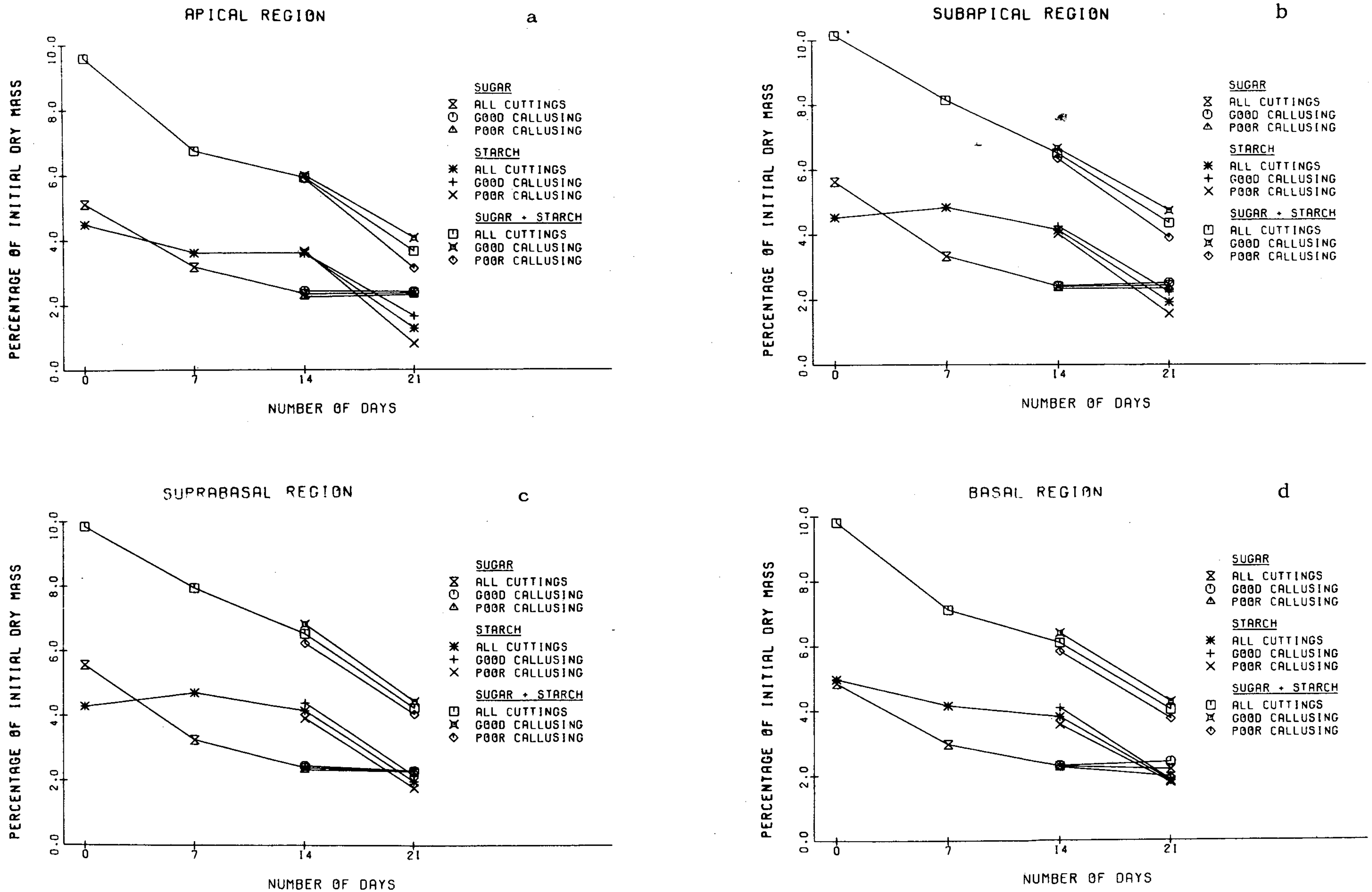


FIG. 18 - Effect of callusing period on sugar and starch concentration of initial dry mass in the apical (a), subapical (b), suprabasal (c), and basal (d), regions of Salt Creek cuttings

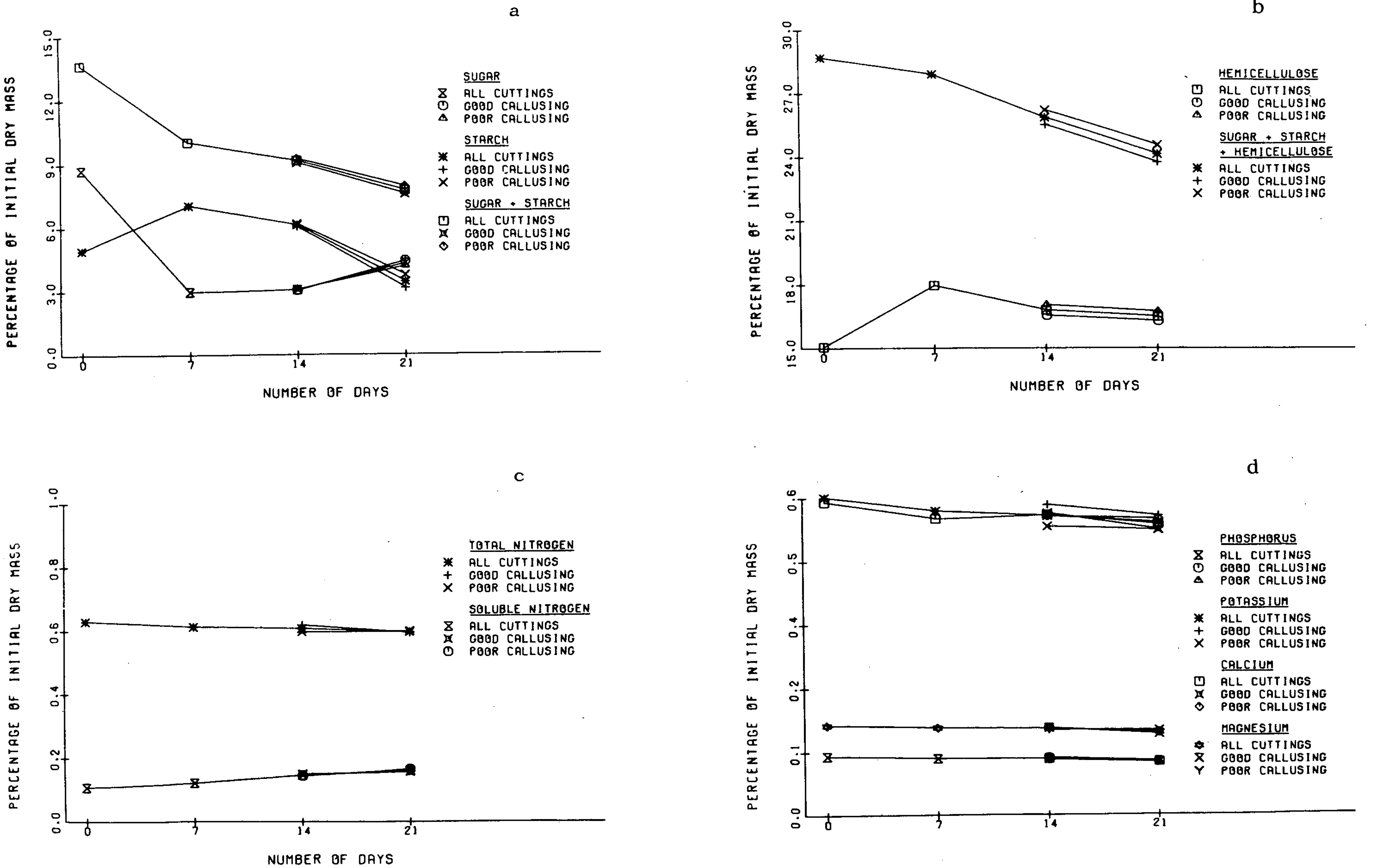


FIG. 19 - Effect of callusing period on sugar and starch (a), hemicellulose and sugar + starch + hemicellulose (b), total N and soluble N (c), phosphorus, potassium, calcium and magnesium (d), concentration of initial dry mass in Waltham Cross scions

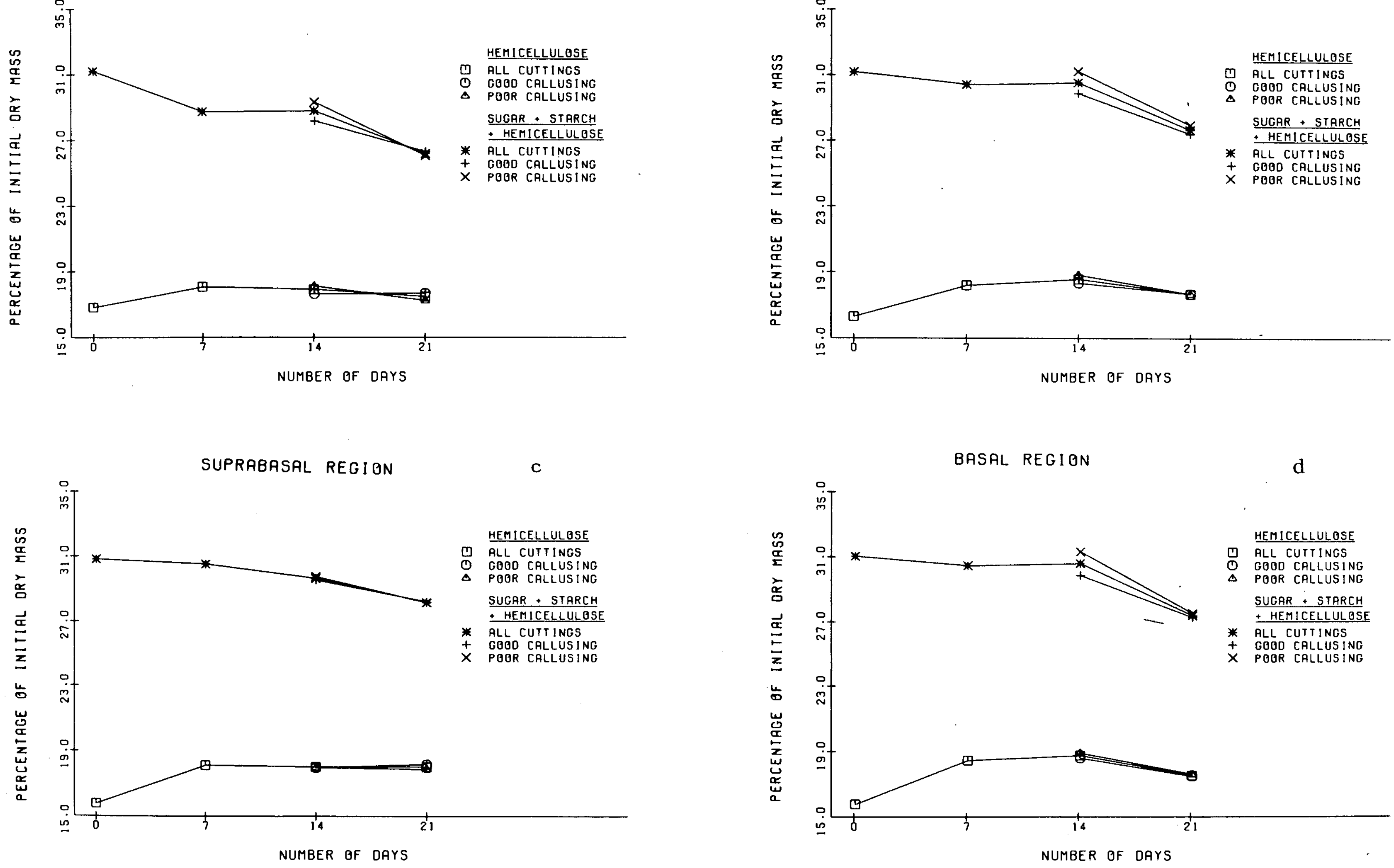


FIG. 20 - Effect of callusing period on hemicellulose and sugar + starch + hemicellulose concentration of initial dry mass in the apical (a), subapical (b), suprabasal (c), and basal (d), regions of Jacquez cuttings

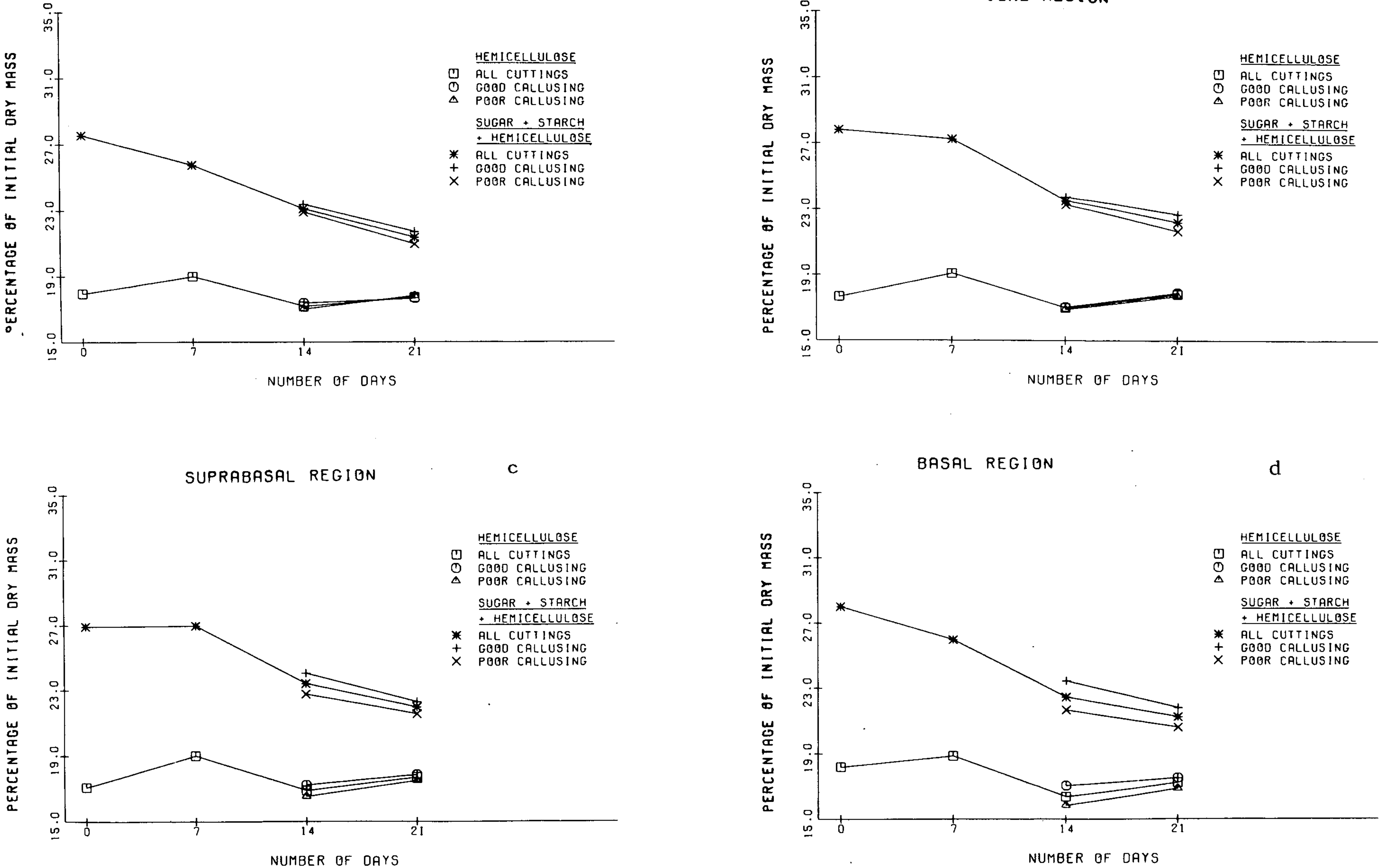


FIG. 21 - Effect of callusing period on hemicellulose and sugar + starch + hemicellulose concentration of initial dry mass in the apical (a), subapical (b), suprabasal (c), and basal (d), regions of Salt Creek cuttings

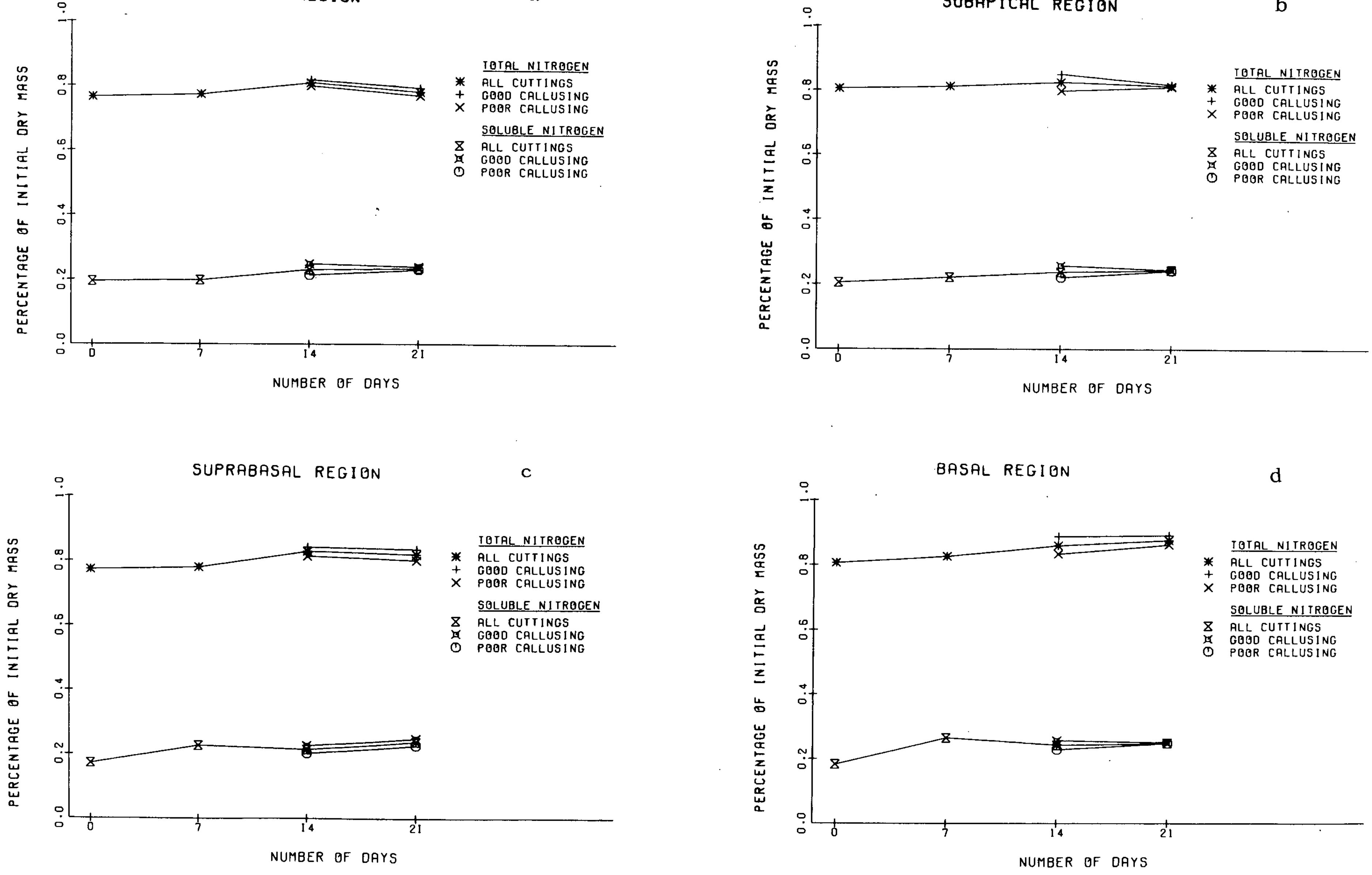


FIG. 22 - Effect of callusing period on total N and soluble N concentration of initial dry mass in the apical (a), subapical (b), suprabasal (c), and basal (d), regions of Jacquez cuttings

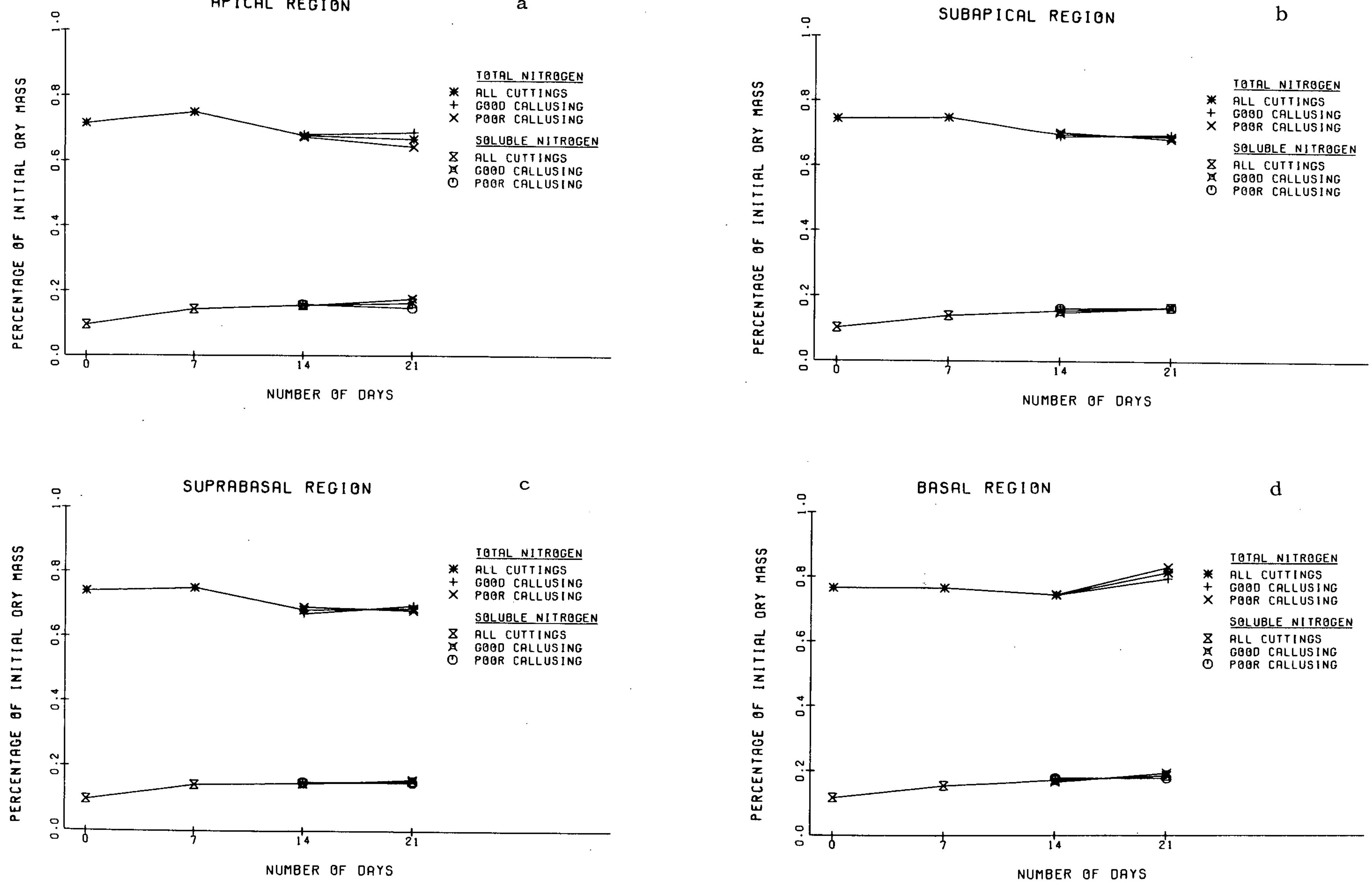


FIG. 23 - Effect of callusing period on total N and soluble N concentration of initial dry mass in the apical (a), subapical (b), suprabasal (c), and basal (d), regions of Salt Creek cuttings

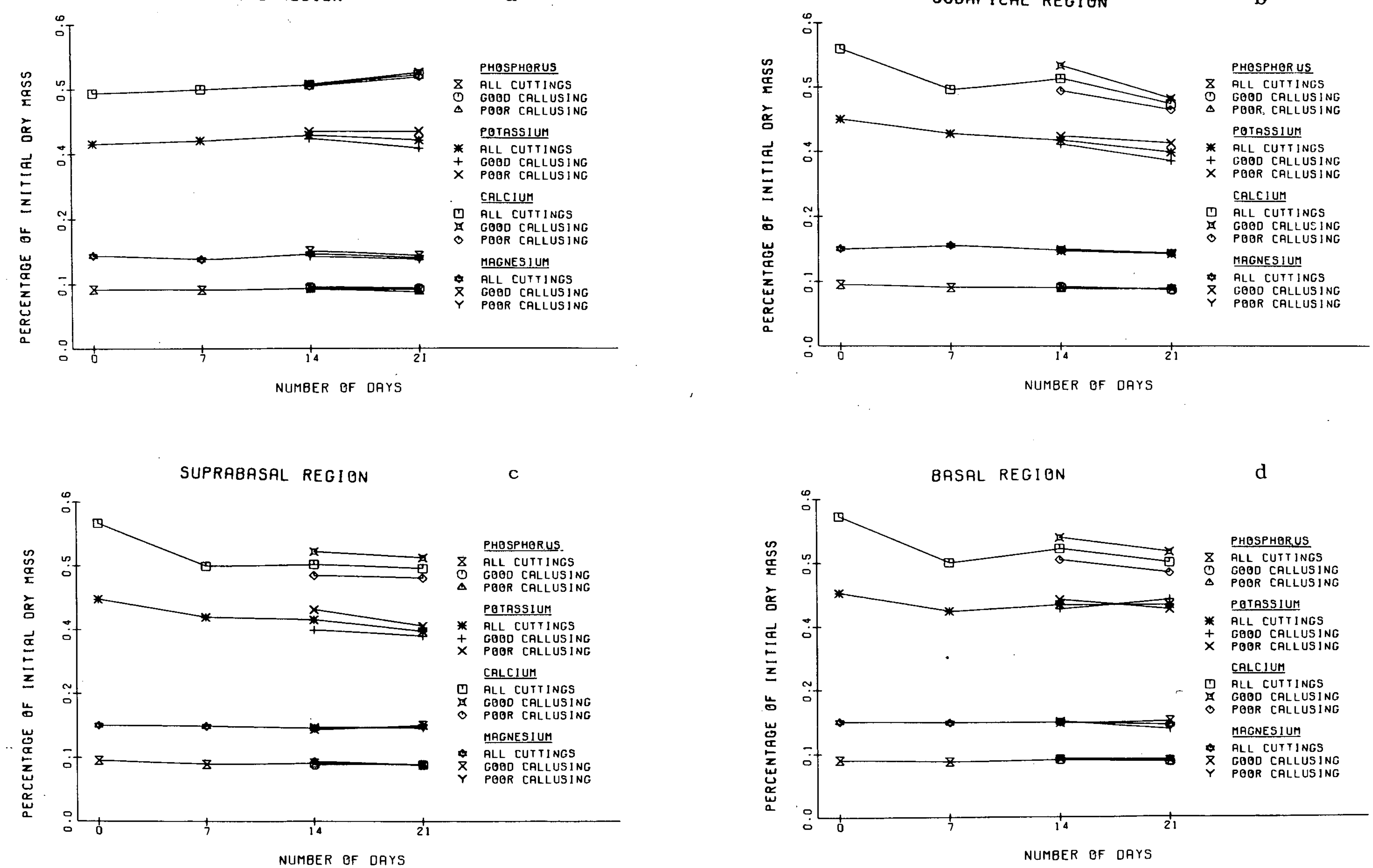


FIG. 24 - Effect of callusing period on phosphorus, potassium, calcium and magnesium concentration of initial dry mass in the apical (a), subapical (b), suprabasal (c), and basal (d), regions of Jacquez cuttings

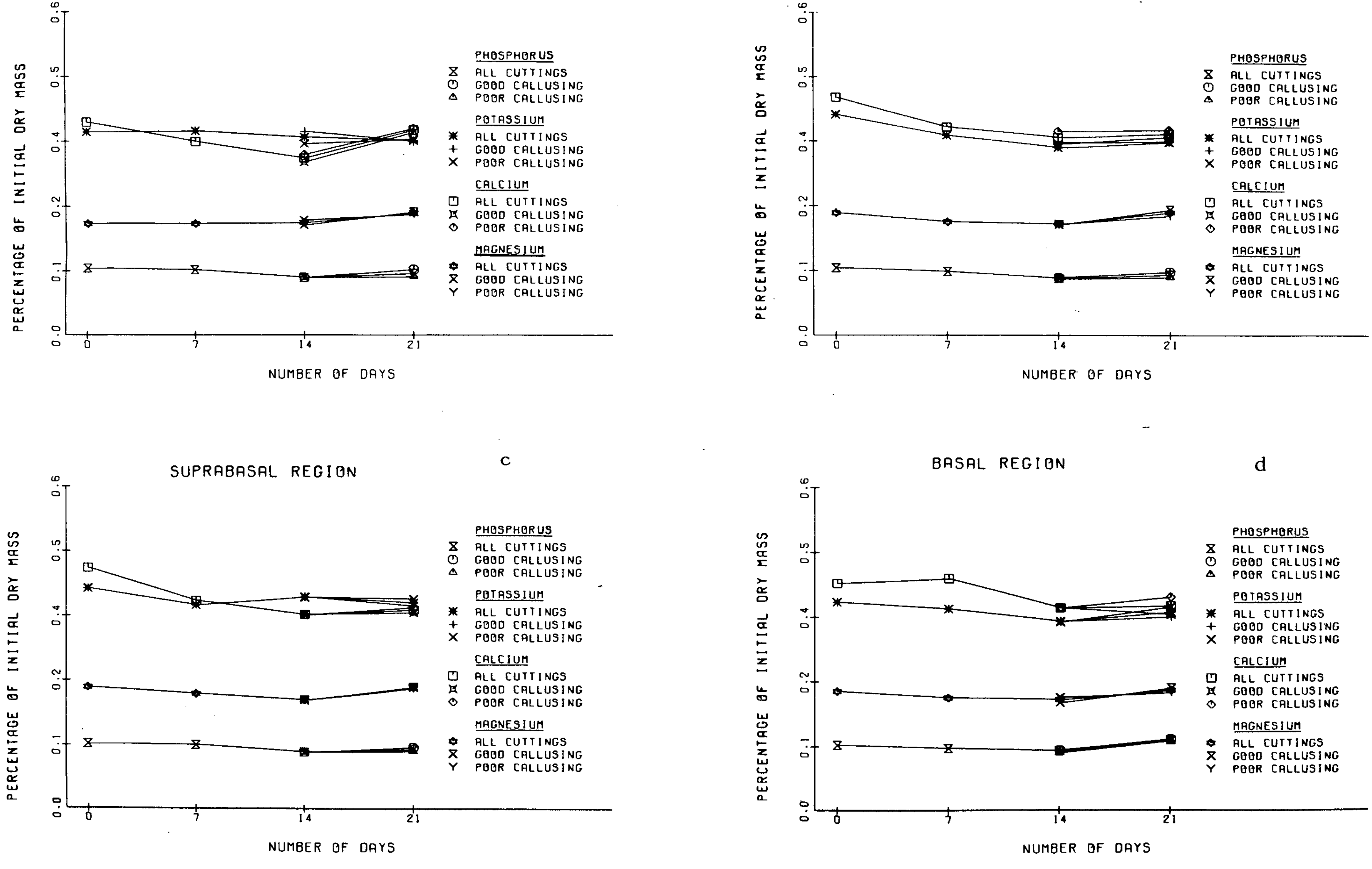


FIG. 25 - Effect of callusing period on phosphorus, potassium, calcium and magnesium concentration of initial dry mass in the apical (a), subapical (b), suprabasal (c), and basal (d), regions of Salt Creek cuttings

TABLE 4 - Effect of callusing period on callusing of Jacquez and Salt Creek cuttings and Waltham Cross scions

Cultivar	Position	Callus score		
		Callusing period (days)		
		7	14	21
Jacquez	Apex	a 0, 1	c 17, 0	c 24, 1
	Base	a 0, 1	b 13, 2	b 20, 0
Salt Creek	Apex	a 0, 2	a 2, 0	a 9, 3
	Base	a 1, 9	e 23, 4	c 26, 0
Waltham Cross	Apex	a 0, 0	a 5, 0	a 8, 0
	Base	b 6, 0	d 21, 6	c 26, 5

a - e : Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

from the callusing room and placed in a Percival growth cabinet where they were kept overnight. The cuttings were therefore held at $25^{o}C$ for 21 days, corresponding with the time callused in section 6. On 14 September the recordings for CO_2 evolution were made.

Preparation of the cuttings for measurement of CO_2 evolution was done as follows: Cuttings were divided into four regions as described in section 6.2, and the cor= responding regions of each replication were grouped. In order to prevent oxidation of the damaged tissue, the freshly cut edges were sealed with a thin layer of pe= troleum jelly. As very little was used, the effect of this on the dry mass was ignored. The apparatus used consisted of sealed respiration chambers over which air at a constant flow and at the same temperature as the cabinet was pas= sed. The CO_2 concentration of the chamber exhaust was measured in an Uras infra red gas analyser.

The samples were left in the chamber for one hour to stabilise before measure= ments were made. Before and after each group of measurements, the CO_2 content of air flowing through an empty chamber was also determined.

The respiration rate was calculated using the following formula:

$$B \quad x \quad \frac{CO_2}{g} \quad x \quad L \quad x \quad \frac{273}{(273\ +\ t)} \quad x \quad \frac{Patm}{101,3} \quad x \quad 1,963$$

Where B = Slope of standard curve

CO_2 = ppm CO_2 released (CO_2 reading - CO_2 air)

L = Flow rate of air (ml. s^{-1})

t = Temperature in ^oC

Patm = Prevailing air pressure (Kpa)

1, 963 = mg CO_2 per ml at STP (0^oC; 101, 3 KPa)

g = g material

7.3 Results and discussion

The respiration rates of four regions of Jacquez and Salt Creek cuttings after callusing for 21 days are presented in Table 5.

In both cultivars the AR and BR showed significantly higher rates than the middle regions. As sugars are needed for respiration and as it was shown in section 6 that the sugar + starch + hemicellulose and sugar losses from the initial value did not differ very much between the regions, there must have been a translocation of sugars from the middle regions of the cuttings to the AR and BR to uphold the higher respiration rates found in these parts.

The only other significant difference was the lower rate in the AR of Salt Creek com= pared with Jacquez.

TABLE 5 - Respiration rate of four regions Jacquez and Salt Creek cuttings after callusing at 25 $^{\circ}$C for 21 days

Region	Respiration rate (ng $CO_2 . s^{-1} . g$ dry mass^{-1})	
	Jacquez	Salt Creek
AR	b 43,7 B	b 37,2 A
SAR	a 32,6 A	a 31,9 A
SBR	a 31,0 A	a 31,4 A
BR	c 48,1 A	c 50,0 A

a - c : Compare in the same vertical column

A, B : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level.

SECTION 8

EFFECT OF CALLUSING TEMPERATURE ON DEGREE OF CALLUSING AND CARBOHYDRATE UTILISATION

8.1 Introduction

Although the technique of callusing grafts in a heated callusing room is widely applied, callusing is still often done at ambient temperatures during spring. It was therefore considered necessary to compare the changes in carbohydrate fractions at different temperatures and to determine whether a relationship exists between these changes and the amount of callus formed at different temperatures. It was shown in section 6 that holding the cutting at 25^{o}C caused an initial conversion of sugar to starch in the Jacquez cuttings and Waltham Cross scions. The question arises as to whether this also takes place at lower callusing temperatures. The following study was undertaken to investigate these aspects.

8.2 Procedure

Cuttings for callusing of Jacquez and Salt Creek were collected at random at the beginning of August 1978, and held at 0^{o}C until they were transferred to the cal= lusing room on 23 August. The experiment was laid out as a factorial design with two factors. These were cultivars (2 levels) and treatments (4 levels). For each combination there were 6 replicates of 20 cuttings each. The four treat= ments consisted of an uncallused control as sampled initially, and the three cal= lusing treatments applied at 15^{o}, 20^{o} and 25^{o}C respectively.

The initial mass of the cuttings in each replicate was determined. Callusing was carried out for 28 days in order to provide the cuttings at the lower temperatures with a prolonged callusing period. After this period the callus was scored and the samples analysed for sugar, starch and hemicellulose.

8.3 Results and discussion

The effect of callusing temperature on the callusing of Jacquez and Salt Creek cut= tings after 28 days is presented in Table 6.1.

Callusing was best at 25°C, slightly poorer at 20°C, while none occurred at 15°C. Thus, within this temperature range the degree of callusing was positively related to temperature. In both cultivars, callusing at the apex was very much poorer than at the base. Jacquez showed a tendency towards better callusing at the apex and poorer callusing at the base when compared with Salt Creek. This last ob= servation was also made in section 6.3.1.

The effect of callusing temperature on sugar, starch and hemicellulose concentration of initial dry mass after callusing for 28 days, is presented in Table 6.2.

The largest losses over the 28-day period were in sugar, and this occured to a greater extent at the higher than the lower temperatures. Starch in Jacquez showed significant increases at all three temperatures. Salt Creek showed an increase at the lower temperatures and a decrease at the highest temperature. From this it can be deduced that sugar-to-starch conversion took place at the lower tempera= tures. Hemicellulose was not significantly affected by callusing or callusing temperature. When interpreting these results it must be borne in mind that they represent one point on a graph such as was shown in section 6.3.2, and that the temperature effects found here are determined by how far the changes in starch and hemicellulose values, which may increase at first before decreasing, had progressed at that temperature.

The sugar + starch + hemicellulose total, which was shown in section 6.3.2. to decrease consistently throughout callusing, gives a clearer indication of the changes that took place. The reduction in this total was greater at the higher than at the lower temperatures. This loss was therefore probably related to respiratory activity. Salt Creek showed a lower percentage loss of sugar + starch + hemicel= lulose at the two lower temperatures compared with that of Jacquez. At 25°C the percentage loss was more or less the same in both cultivars. This result is again referred to in section 11.

The relationships between reduction in percentage of sugar + starch + hemicellulose and callus score at the apex of Jacquez, and the apex and base of Salt Creek are presented in Figs. 26a; 27a, b respectively. The linear regression line between the same two factors for the base of Jacquez is presented in Fig. 26. This re= gression coefficient was significant. There was therefore a tendency for the reduction in sugar + starch + hemicellulose content to increase with incease with increasing callus formation. It therefore seems that these carbohydrates played an important role in callus formation.

TABLE 6.1 - Effect of callusing temperature on callusing of Jacquez and Salt Creek cuttings after 28 days

Position	Rootstock	Callus score		
		Callusing temperature (°C)		
		15°C	20°C	25°C
Apex	Jacquez	0,00 A	0,04 A	5,43 B
	Salt Creek	0,00 A	0,00 A	1,24 A
Base	Jacquez	0,00 A	16,25 B	21,33 C
	Salt Creek	0,00 A	19,46 B	23,51 C

TABLE 6.2 - Effect of callusing temperature on sugar, starch and hemicellulose as concentration of initial dry mass in Jacquez and Salt Creek cuttings after callusing for 28 days

Component	Rootstock	Percentage of initial dry mass				Percentage change from initial value		
		Initially	Callusing temperature (°C)			Callusing temperature (°C)		
			15°C	20°C	25°C	15°C	20°C	25°C
Sugar	Jacquez	9,78 C	3,89 B	2,93 A	3,06 A	- 60,2	- 70,0	- 68,7
	Salt Creek	6,40 C	3,33 B	2,92 AB	2,63 A	- 48,0	- 54,4	- 58,9
Starch	Jacquez	6,30 A	9,12 B	8,22 B	8,02 B	+ 44,8	+ 30,5	+ 27,3
	Salt Creek	4,74 A	6,75 B	5,82 AB	4,40 A	+ 42,4	+ 22,8	- 7,17
Sugar + starch	Jacquez	16,08 C	13,01 B	11,15 A	11,08 A	- 19,1	- 30,6	- 31,1
	Salt Creek	11,14 C	10,08 BC	8,74 B	7,03 A	- 9,5	- 21,5	- 36,9
Hemicellulose	Jacquez	16,31 A	16,64 A	16,43 A	15,71 A	+ 2,02	+ 0,74	- 3,68
	Salt Creek	16,64 A	16,20 A	16,15 A	15,81 A	- 2,64	- 2,94	- 4,98
Sugar + starch + hemicellulose	Jacquez	32,39 C	29,65 B	27,58 AB	26,79 A	- 8,46	- 14,8	- 17,3
	Salt Creek	27,78 C	26,28 B	24,89 B	22,84 A	- 5,40	- 10,4	- 17,8

A - C : Compare in the same horizontal row. Means accompanied by a common letter are not different at the 5% level

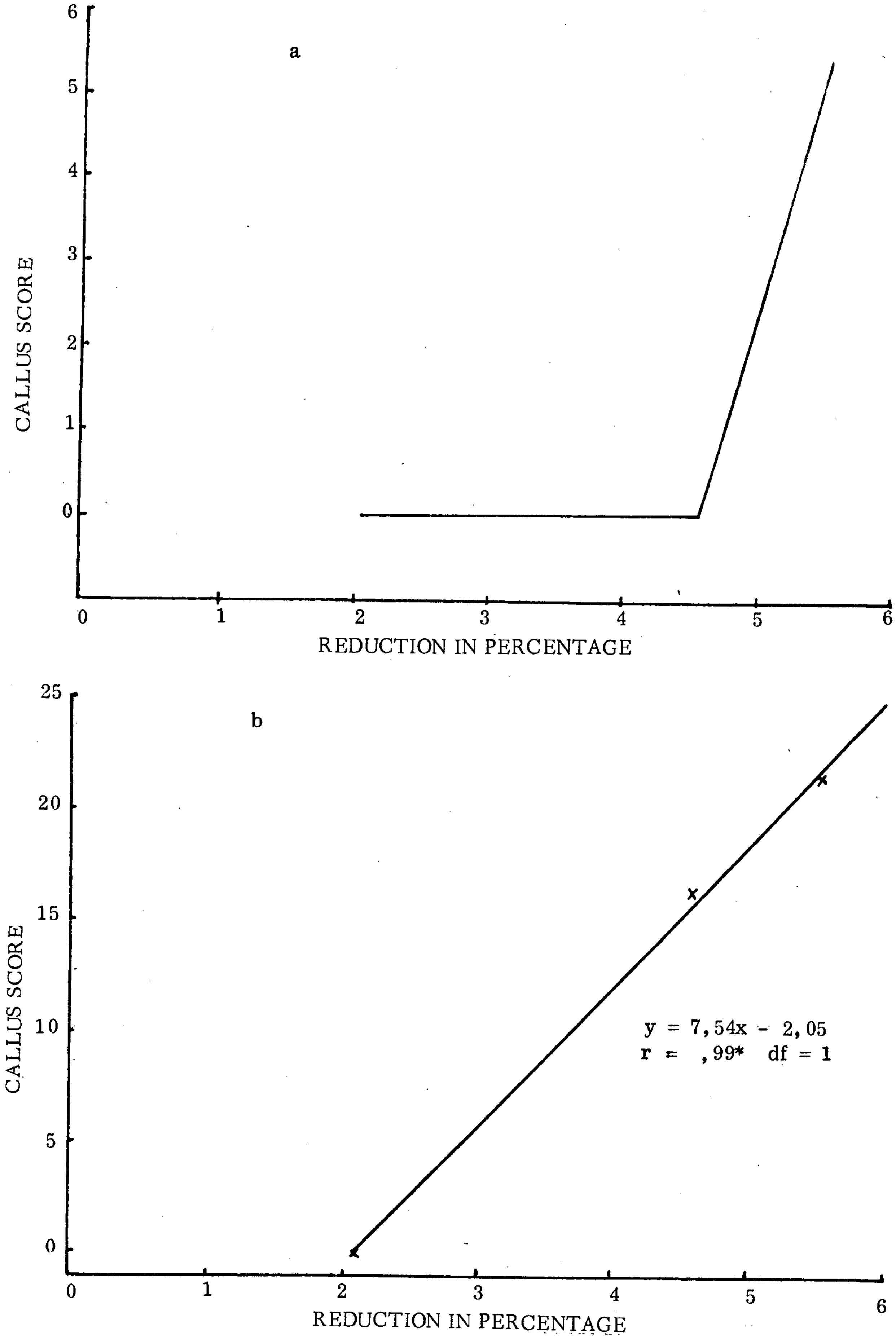


Fig. 26 - (a) Relation between callus score at the apex and reduction in percentage sugar + starch + hemicellulose of Jacquez cuttings

(b) Linear regression line between callus score and reduction in percentage of sugar + starch + hemicellulose at the base of Jacquez cuttings

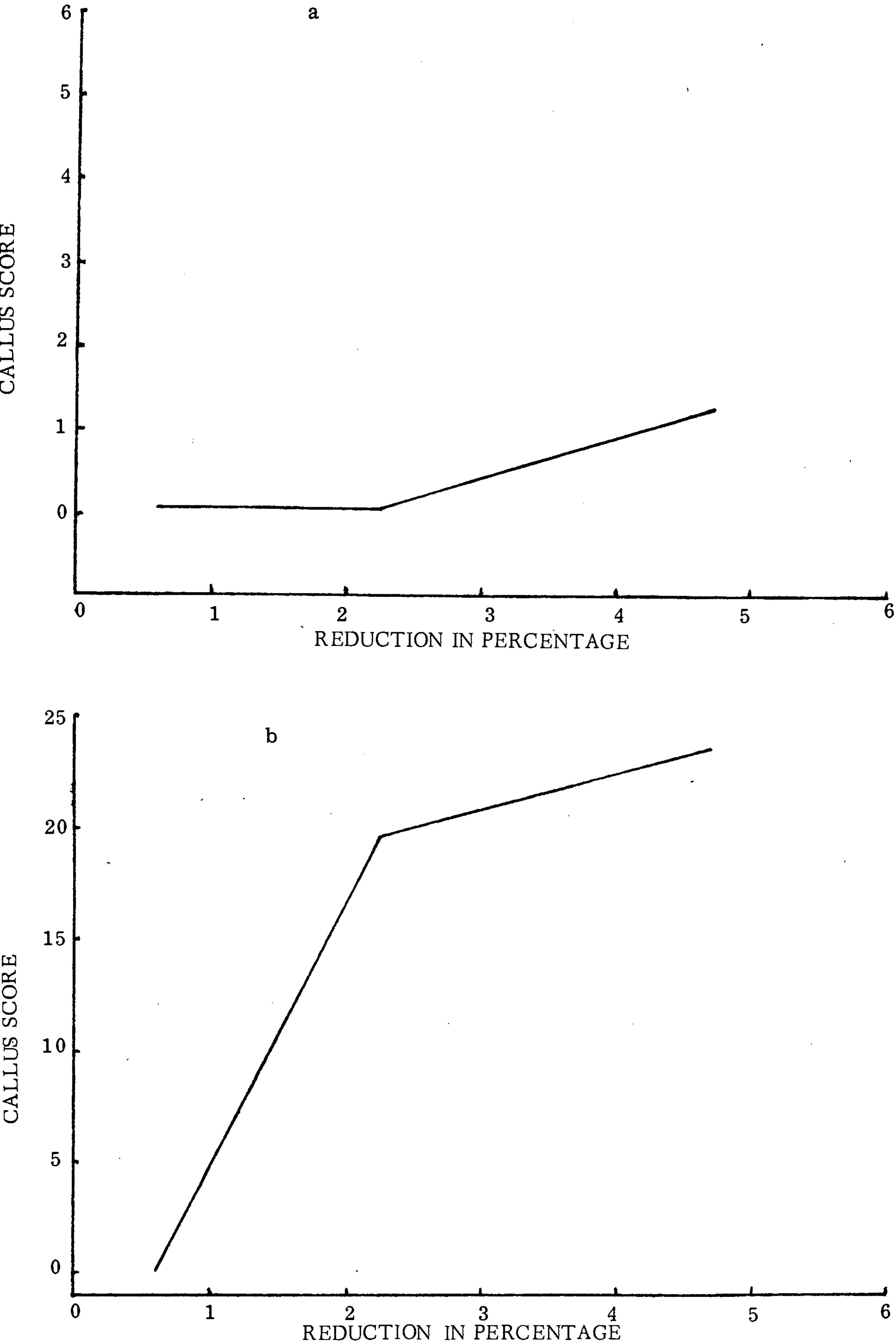


Fig. 27 – Relation between callus score at the apex (a) and base (b) and reduction in percentage of sugar + starch + hemicellulose of Salt Creek cuttings

SECTION 9

EFFECT OF CUTTING LENGTH ON CALLUSING AND CARBOHYDRATE UTILISATION

9.1 Introduction

Rootstock cuttings for grafting are usually about 25 cm in length; this includes several nodes. The successful use of shorter rootstock cuttings would be an advantage where rootstock material is in short supply as is the case for new virus-free clones. As the shorter cutting would also contain less carbohydrates, it is important to know at which cutting length available carbohydrates would be= come a limiting factor to callus formation. This aspect was studied in this experiment. In addition, the effect of the presence of buds on callusing was also investigated.

9.2 Procedure

Cuttings for callusing of Jacquez and Salt Creek were collected at random at the beginning of August 1978 and held at $0^{o}C$ until the start of the experiment on 23 August.

The experiment was laid out using a factorial design. There were two factors namely cultivar (2 levels) and treatment (9 levels). The treatments were three controls and six callused treatments as follows:-

Cutting lengths

Cutting lengths		
20 - 25 cm with buds	Controls	Callused
8 cm with one bud in the middle	Controls	Callused
8 cm internode	Controls	Callused
4 cm internode		Callused
2 cm internode		Callused
1 cm internode		Callused

Six replicates of 20 cuttings were used for each treatment. The initial mass of the cuttings in each replicate was determined. In order to allow the shorter cut= tings to callus sufficiently, callusing was done for 28 days. At the end of the period the callus was scored and samples taken for analysis. All samples were analysed for sugar, starch and hemicellulose. Analytical results were expressed as concentration of dry mass or initial dry mass. For determination of the re= gression coefficients an average length of 22,5 cm was used for the 20 - 25 cm cuttings.

9.3 Results and discussion

The effects of cutting length and the presence of a bud on the sugar, starch and hemicellulose expressed as concentration of dry mass in Jacquez and Salt Creek cuttings initially, and as concentration of initial dry mass after callusing for 28 days, are presented in Table 7.1. The effects of the same factors on the callusing in these cuttings are presented in Table 7.2.

The slight difference in initial sugar and starch concentration between the 8 cm cuttings with and without buds can probably be ascribed to differences in concentration between node and internode. Only in the case of sugar in Salt Creek was this difference significant. In this regard, Georgescu and Ilie (1970) found that more starch accumulated in the nodes than in the internodes during autumn and spring.

The most important changes in sugar, starch and hemicellulose during callusing were as follows. The largest loss was in sugar, with little difference in the final concentration between cutting lengths. In Jacquez, starch concentration de= creased significantly only in the 1 cm cuttings. Starch concentration in Salt Creek decreased significantly in the 4, 2 and 1 cm cuttings and the shorter the cuttings, the lower was the final starch concentration. Nevertheless, even in the 1 cm cutting, there was starch remaining after 28 days of callusing. There appeared to be a positive relationship between the length of the cuttings and sugar + starch concentration after callusing. Therefore, the shorter the cutting the larger the percentage sugar + starch which was utilised.

There was little change in the hemicellulose of Jacquez during callusing and the final values did not differ statistically from each other or from the initial values. Salt Creek generally showed small losses of hemicellulose during callusing. The final value in the 4 cm cuttings differed significantly from the initial value.

Except for the higher concentration of sugar + starch + hemicellulose in the 8 cm cuttings with a bud, compared to the 20 - 25 cm cuttings, the final concentration in both cultivars decreased with decreasing cutting length. There were no sig= nificant differences in concentration between the 8 cm cuttings, with and without a bud, for any of the components. The presence of a bud had no influence on the utilisation of sugar and starch.

Cutting length had little influence on callusing at the apex. The one significant difference, that between the 8 cm cutting with a bud and the 4 cm internode, seems to have been caused by the presence of a bud rather than the cutting length. The 8 cm cutting with a bud callused less at the apex than the 8 cm internode, but the dif= ference was not statistically significant.

Callusing at the base of both cultivars was significantly influenced by cutting length. In an attempt to explain this phenomenon the callus score / cutting length (Fig. 28) and callus score / reduction in carbohydrates (Fig. 29) relation= ships were considered. These relationships showed basically the same response phenomenon, namely an almost linear increase in callus score with the other two independent variables up to 8 cm length, beyond which the response levelled off. These results therefore suggest that cutting length could be a limiting factor in callus formation at the base, due to its level of available carbohydrates, and that 22, 5 cm long cuttings contained carbohydrates at a luxury level, in respect of callus formation.

The 8 cm cuttings with or without a bud did not differ significantly with regard to callusing at the base, and it can be concluded that the presence of a bud had no influence on callusing performance at the base.

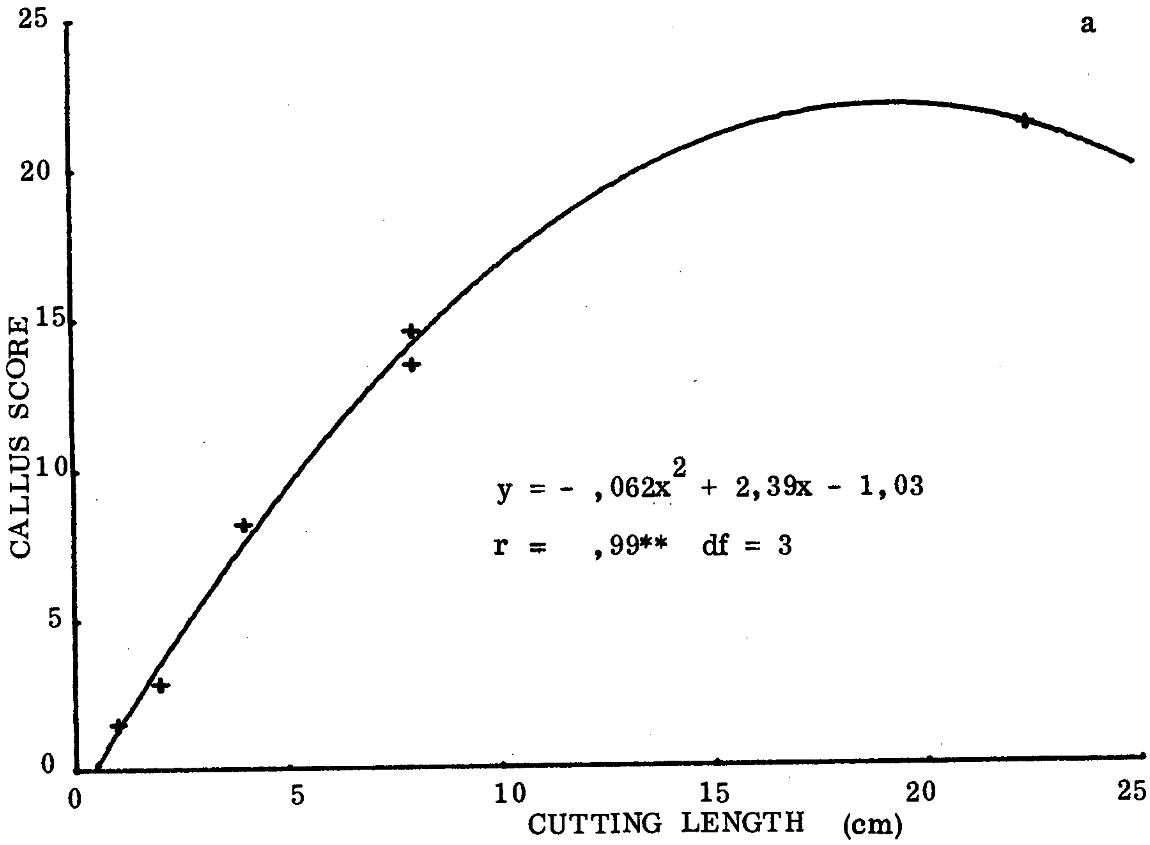
TABLE 7.1 - Effect of cutting length and the presence of a bud on sugar, starch and hemicellulose as concentration of dry mass in Jacquez and Salt Creek cuttings initially and as concentration of initial dry mass after callusing for 28 days

Component	Rootstock	Concentration of components %								
		Initially			Callused					
		20-25 cm with buds	8 cm with bud	8 cm internode	20-25 cm with buds	8 cm with bud	8 cm internode	4 cm internode	2 cm internode	1 cm internode
Sugar	Jacquez	9,78 B	10,89 C	10,86 C	3,06 A	2,73 A	2,82 A	2,82 A	2,65 A	2,38 A
	Salt Creek	6,40 D	6,52 D	7,16 E	2,63 BC	2,68 BC	2,76 C	2,42 BC	2,04 AB	1,65 A
Starch	Jacquez	6,30 D	5,91 CD	5,07 BC	8,02 E	6,55 D	5,95 CD	5,17 BC	4,23 BC	3,72 A
	Salt Creek	4,74 B	4,74 B	3,95 B	4,40 B	3,98 B	3,89 B	2,71 A	2,22 A	1,77 A
Sugar + starch	Jacquez	16,08 E	16,80 E	15,93 E	11,08 D	9,28 C	8,77 BC	7,99 B	6,88 A	6,10 A
	Salt Creek	11,14 D	11,26 D	11,11 D	7,03 C	6,66 C	6,65 C	5,13 B	4,26 AB	3,42 A
Hemicellulose	Jacquez	16,31 A	15,69 A	15,31 A	15,71 A	15,95 A	15,05 A	14,43 A	15,00 A	15,61 A
	Salt Creek	16,64 BC	16,92 C	16,73 BC	15,81ABC	16,70 BC	15,03ABC	14,44 A	14,82 AB	15,60ABC
Sugar + starch + hemicellulose	Jacquez	32,39 D	32,49 D	31,24 D	26,79 C	25,23 BC	23,82 AB	22,42 A	21,88 A	21,71 A
	Salt Creek	27,78 C	28,18 C	27,84 C	22,84 B	23,36 B	21,68 B	19,57 A	19,08 A	19,02 A

TABLE 7.2 - Effect of cutting length and the presence of a bud on the callusing of Jacquez and Salt Creek cuttings after 28 days

Position	Rootstock	Callus score					
		20-25 cm with buds	8 cm with bud	8 cm internode	4 cm internode	2 cm internode	1 cm internode
Top	Jacquez	5,44 AB	1,97 A	6,64 AB	8,63 B	4,46 AB	3,42 AB
	Salt Creek	1,04 A	4,11 A	3,35 A	2,01 A	2,65 A	0,33 A
Bottom	Jacquez	21,33 D	14,60 C	13,48 C	8,21 B	2,89 A	1,50 A
	Salt Creek	23,51 D	15,69 C	16,98 C	8,62 B	2,78 A	2,58 A

A - E : Compare in the same horizontal row. Means accompanied by a common letter are not different at the 5% level



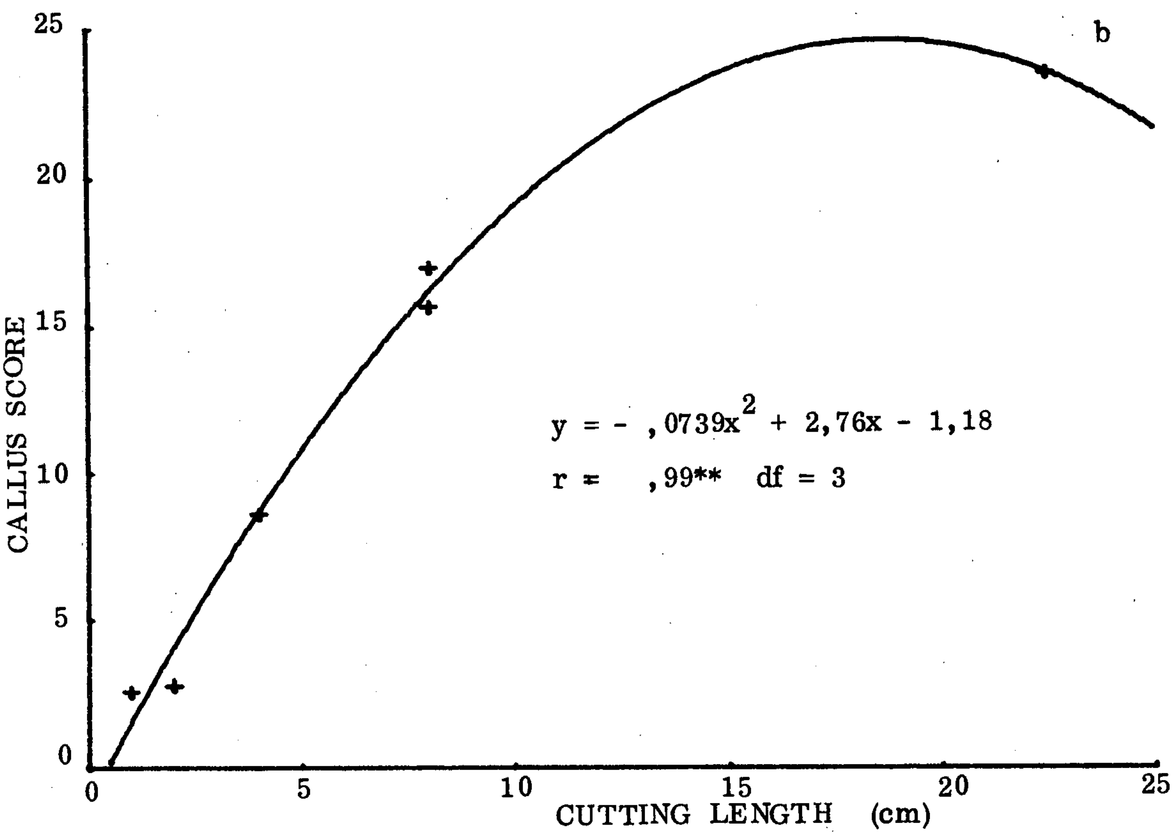
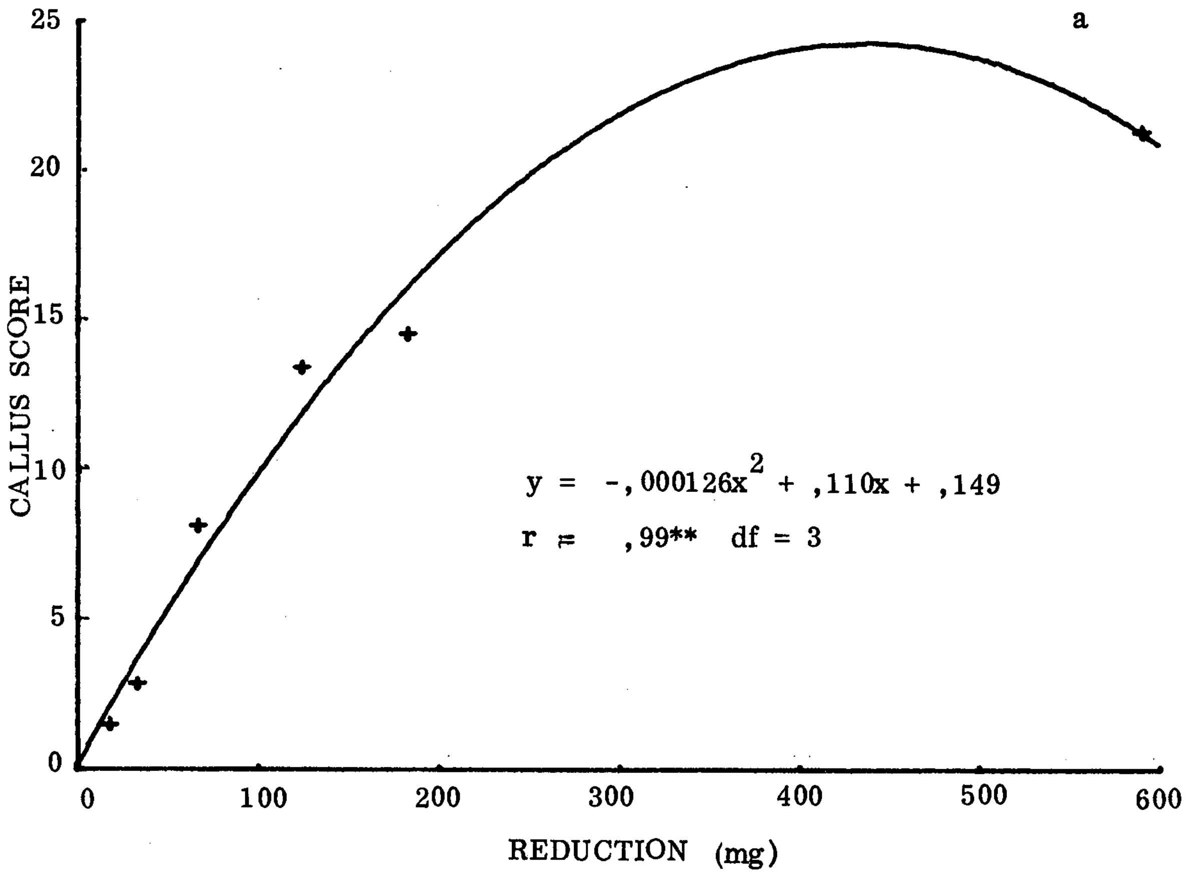


Fig. 28 – Second degree regression between callus score at the base of Jacquez (a) and Salt Creek (b) and cutting length



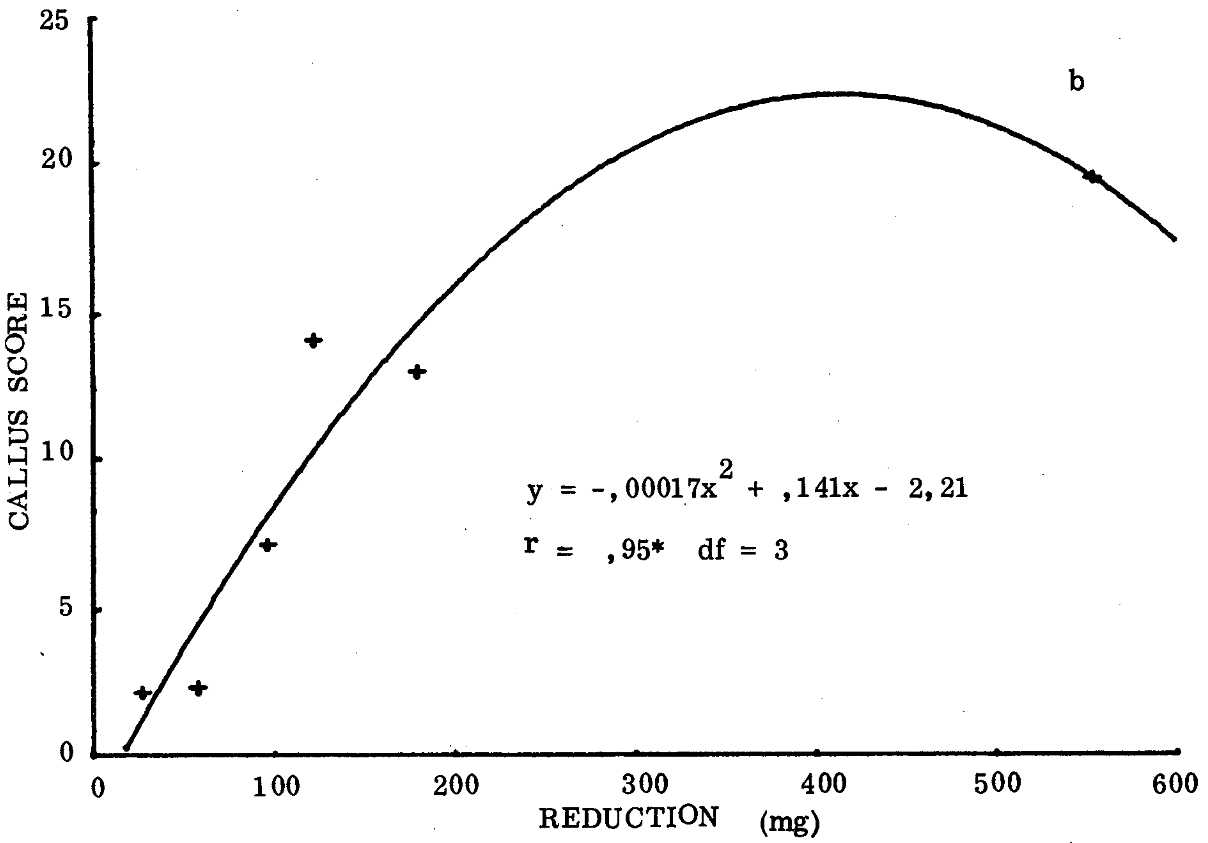


Fig. 29 - Second degree regression between callus score at the base and reduction in sugar + starch + hemicellulose for Jacquez (a) and Salt Creek (b) cuttings

SECTION 10

SEASONAL CHANGES IN CARBOHYDRATE FRACTIONS IN VINES DURING THE NURSERY STAGE

10.1 Introduction

For a variety of deciduous plants it has been shown that the development of the cutting during propagation viz. the formation of roots and shoots is, among other factors, dependent on their stored reserves of carbohydrates (Buttrose, 1966 for grapes; Stassen, 1973 for apples; Brutsch, 1971 and Smith, 1972 for pecans).

However, this phonomenon has never been quantified for Jacquez and Salt Creek rootstock cuttings. Since these two rootstocks are regarded as very important for the South African table grape industry, this study and those in sections 11 and 12 were undertaken to obtain the necessary information.

10.2 Procedure

Cuttings for rooting of Jacquez and Salt Creek were collected at random at the beginning August 1975. They were stored in plastic bags at $0^{o}C$ until 19 August when they were planted.

The experiment was laid out as a factorial arrangement of treatments. There were two factors namely cultivar (Jacquez and Salt Creek) and sampling dates (19 August; 2, 16 and 30 September; 14, 28 October; 11, 25 November; 22 December, 22 January, 23 February, 22 March, 24 May and 23 July). The factors were replicated in 6 randomized blocks with 10 cuttings per replicate. Cuttings for sampling dates from 2 September onwards were planted on 19 August according to the procedure described in section 3.5. When sampling the planted cuttings, the plants were removed from the medium according to the procedure described in section 3.5. They were then washed, divided into roots, stem, shoots and leaves (when applicable) and these portions prepared for analysis.

The samples were analysed for sugar, starch and hemicellulose. Results were expressed as concentration of dry mass as well as on an absolute basis (g/plant).

10.3 Results and discussion

A cutting becomes the stem of the plant as soon as it has developed roots, shoots and leaves. In order to avoid confusion, only the term "stem" will be used for the purpose of this discussion and will therefore also refer to the "cutting". Si= milarly, when mention is made of the whole plant, the term "plant" will be used and this will also refer to the "cutting" before development of roots, shoots and leaves.

10.3.1 Dry mass

The seasonal changes in dry mass during the growth of Jacquez and Salt Creek plants are presented in Figs. 30a, 31a and Appendix 33.1.

The stems of both cultivars showed a loss in dry mass during the first twelve weeks, but their dry mass started increasing after 11 November, reaching a final value on 23 July which was only slightly higher than the initial mass of the cutting.

Shoot and leaf growth started before root growth, but the final dry mass of the roots was much higher than that of the shoots, the ratio being about 3,2:1 for Jacquez and 1,7:1 for Salt Creek. Salt Creek showed considerably more shoot growth than Jacquez.

In both cultivars the leaf dry mass increased until 22 March and until that time it was always higher than that of the shoot dry mass. After this date abscission of leaves commenced, starting sooner in Jacquez than in Salt Creek. The tem= porary decrease in leaf dry mass on 23 February is considered to be due to the loss of the smaller older leaves because of hot and dry weather conditions at that stage.

10.3.2 Sugar, starch and hemicellulose

The seasonal changes in sugar, starch and hemicellulose concentration of dry mass during the growth of Jacquez and Salt Creek plants are presented in Figs. 30b - d, 31b - d, 32, 33 and Appendices 33.2 - 33.4.

Initial sugar concentration in both cultivars was higher than that of starch, while the two fractions combined was higher in Jacquez (15,60%) than in Salt Creek (11,84%). These figures compare reasonably well with that recorded by Buttrose (1966) in Sultana and by Hosoi et al. (1972) in Delaware grapes. The changes in the two cultivars were remarkably similar, and unless otherwise stated, they will be discussed together. Heavy losses in sugar occurred in the stem during the first four weeks, which coincided with an increase in starch. After this period, sugar and starch decreased to a minimum around 11 and 25 November.

These cuttings were therefore partly dependent on their sugar and starch reserves for a period of twelve weeks after planting. This period corresponds very well with the results of Schaefer (1978) who, in three graft combinations, recorded minimum values in the sugar plus starch content in the scion and rootstocks after 12 and 14 weeks respectively. After reaching the minimum values, starch in the stem increased rapidly, while the sugar remained constant at just above 3%. The increase in starch concentration coincided with the tremendous expansion of the leaf area starting from 25 November. The excess carbohydrates formed during this period were therefore stored as starch. Although there were signs of some conversion of starch to sugar from 24 May onwards, sugar values in the stem were still lower than those of starch on 23 July.

The increase in starch concentration in the shoots was more pronounced for Jacquez than for Salt Creek. This can be explained by the latter having more shoot growth, which utilised a larger proportion of the carbohydrate production. Following 24 May there was a conversion of starch to sugar in the shoots, resulting in a higher sugar than starch concentration on 23 July. The concentration of sugar + starch in the shoots on this date was higher in Jacquez than in Salt Creek.

Accumulation of starch in the roots took place at the same stage as in the stem, reaching a maximum concentration of 28, 88% in Jacquez and 25, 71% in Salt Creek on 24 May. The slight decrease in starch concentration in Jacquez from 23

February to 22 March and the less pronounced increase in Salt Creek during that time coincided with a period of rapid root growth. Sugar concentration of the roots remained low throughout the season and there was no conversion of starch to sugar during winter. As the soil temperature in the Western Cape is never low enough to produce cold injury, such a conversion is unneccessary in the roots. Winkler and Williams (1945) also found no indication of starch to sugar conversion in the roots during early winter.

The stem, shoots and roots therefore showed a similarity to the mature canes (section 4.3.1.2) in that sugar concentration during summer and autumn, re= mained fairly constant, the excess sugar being converted to starch and other products.

In this study both cultivars initially had higher sugar than starch concentration, whereas Buttrose (1966) recorded initially high starch and low sugar concentra= tions. The initial sugar + starch concentration of dry mass recorded by Buttrose in Sultana (just over 15%) and Hosoi et al. (1972) in Delaware (just under 14%) compares very well with that of Jacquez in this study (15,6%) but was higher than that of Salt Creek (11,8%).

This study showed similarity with that of Buttrose (1966) and Schaefer (1978) in that sugar concentration, once it had reached a low value, remained fairly con= stant.

In section 3.6.1.3 it was explained that a loss of sugar and starch, which make out part of the dry mass, could mask small changes in a substance such as hemi= cellulose if the results are expressed as concentration of dry mass. The correct way of detecting such small changes would be to express results as an absolute amount or as concentration of initial dry mass. This is done later in this section and in section 11, and at this stage only the hemicellulose concentration following 22 December will be discussed.

From this stage onwards, the stem showed a gradual decrease in hemicellulose concentration up to 24 May, coinciding with a sharp increase in starch. The shoots showed a considerable increase in hemicellulose up to 23 February, very similar to that of the mature vine (section 4.3.1.2) and then remained fairly constant until 24 May. Following this date, both the stem and shoots showed a small decrease in hemicellulose, corresponding with the loss recorded in the mature vine (section 4.3.1.2). Compared with the hemicellulose content of stem and shoots, this material did not appear to be an important component in the roots, and it remained constantly low after an initial decrease from 25 Novem= ber to 22 December.

The seasonal changes in sugar, starch and hemicellulose content during growth of Jacquez and Salt Creek plants are presented in Figs. 34, 35 and Appendices 33.5 - 33.7.

Sugar in the stem of both cultivars followed much the same pattern as the concen= tration graph. On the other hand, starch during the first four weeks showed a decrease in Jacquez and a smaller increase in Salt Creek compared to the concen= tration graph. This difference must be regarded as the result of a change in dry mass caused by the sugar loss in the percentage graph. The loss of hemicellulose content showes that some hemicellulose was utilised at the same time as the large sugar and starch losses occurred. Following the lowest levels of starch and hemi= cellulose which occurred almost simultaneously, hemicellulose increased concomitant= ly with the increase in starch. This increase can be regarded as the result of the replenishment of that depleted earlier, as well as the formation of new cell walls in new growth of the stem. It is clear that at least part of the hemicellulose fraction must be regarded as a reserve carbohydrate. In the shoots hemicellulose accumu= lation was very marked, whereas in the roots starch prevailed as the dominating of the three carbohydrate components.

The percentage distribution of the combined values of the two most important reserve carbohydrates in the plant, namely sugar and starch, on 23 July, is indicated below.

	Stem	Shoots	Roots
Jacquez	26,2	9,1	64,7
Salt Creek	24,5	16,2	59,3

These results suggest that excessive removal and shortening of roots on rooted vines prior to planting might be very detrimental of the carbohydrate status of the vine. The effect of severe pruning of canes would most probably be less detrimental.

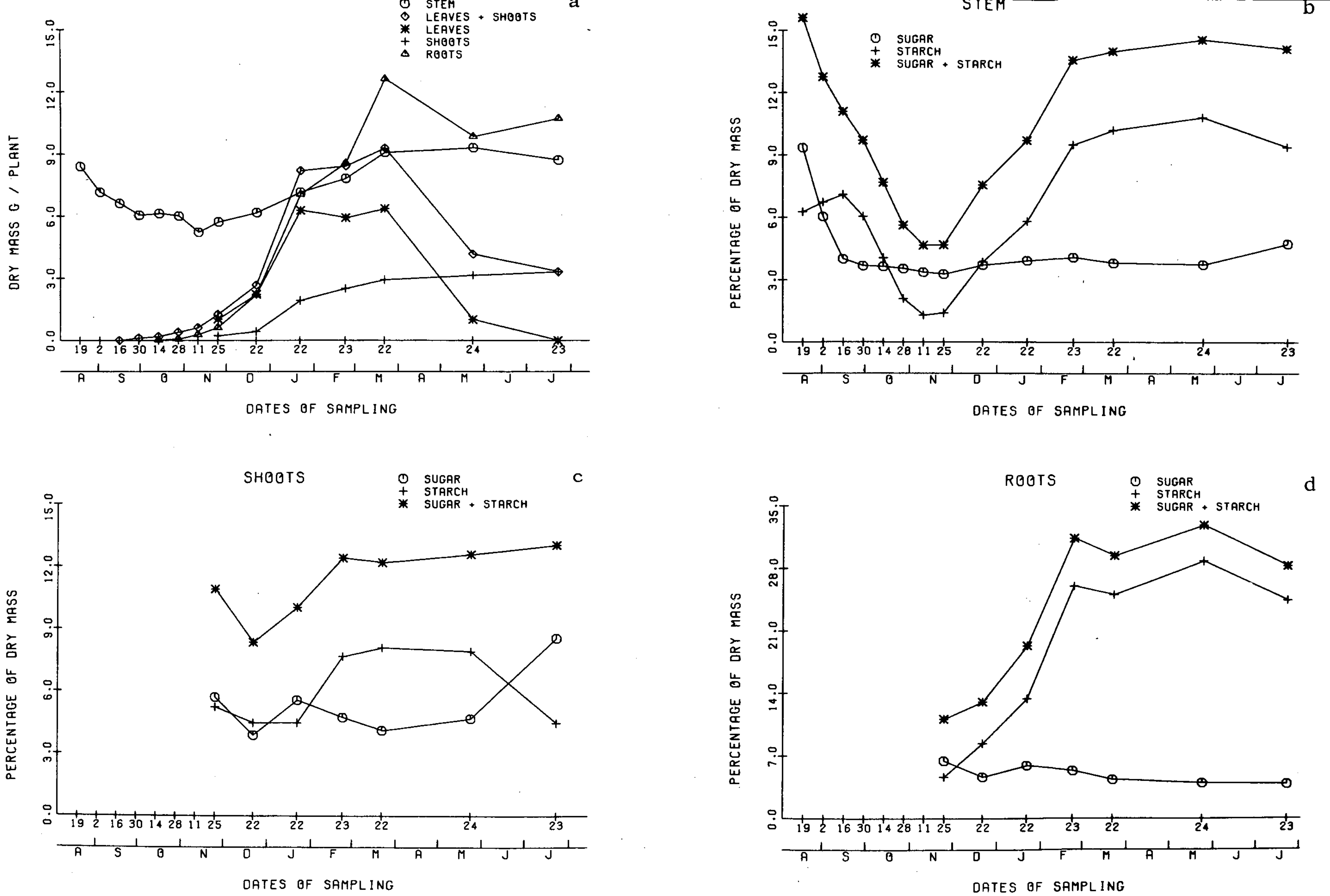


FIG. 30 - Seasonal changes in dry mass of different Jacquez plant parts (a), sugar and starch concentration of dry mass in the stem (b) shoots (c) and roots (d)

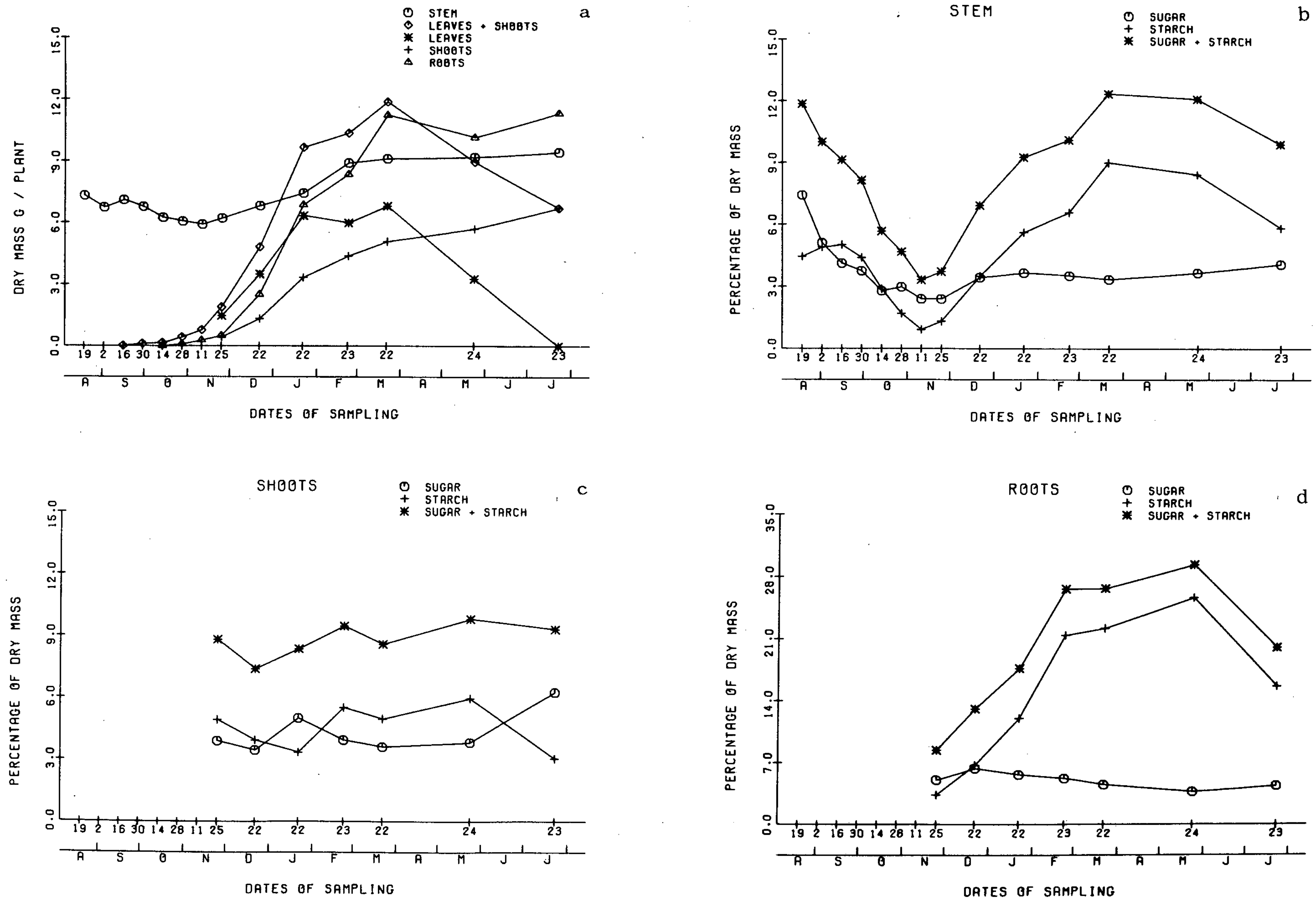


FIG. 31 - Seasonal changes in dry mass of different Salt Creek plant parts (a), sugar and starch concentration of dry mass in the stem (b) shoots (c) and roots (d)

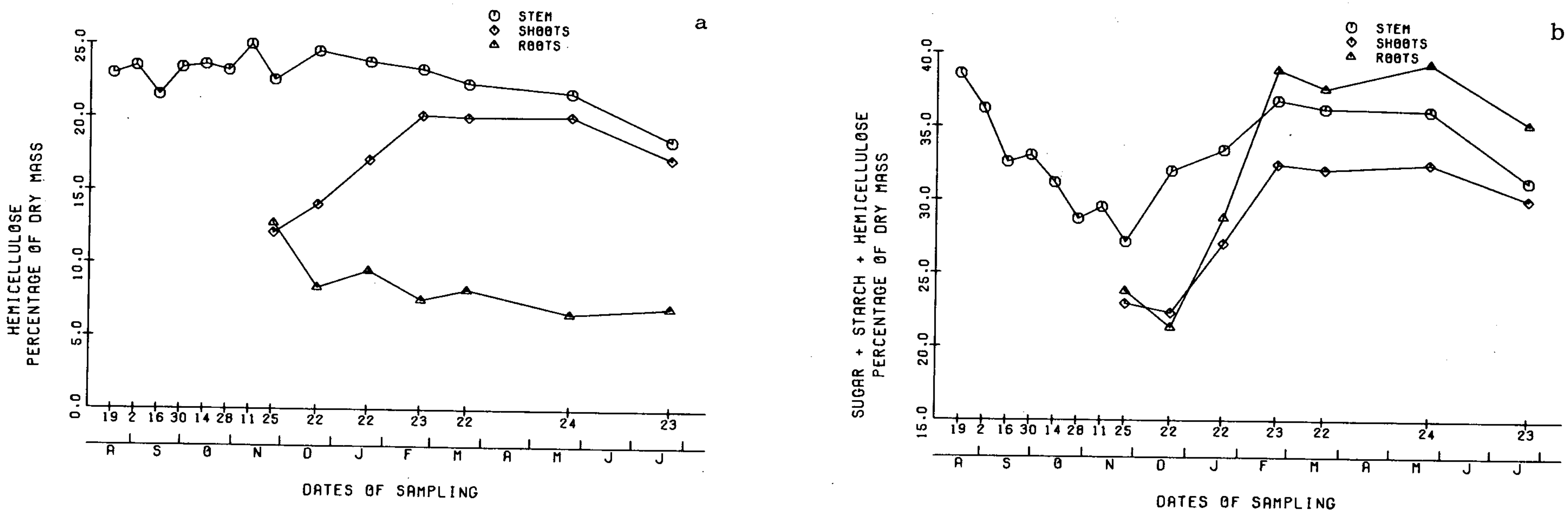


FIG. 32 - Seasonal changes in hemicellulose (a), sugar + starch + hemicellulose (b) concentration of dry mass in different Jacquez plant parts

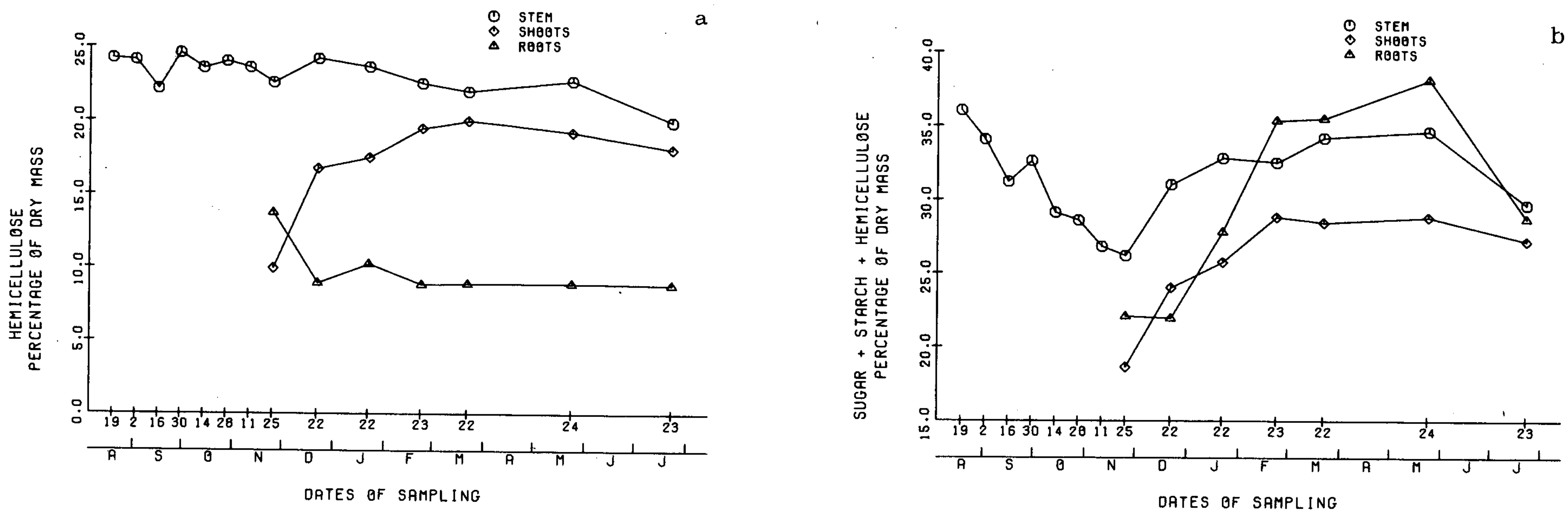


FIG. 33 - Seasonal changes in hemicellulose (a), sugar + starch + hemicellulose (b) concentration of dry mass in different Salt Creek plant parts

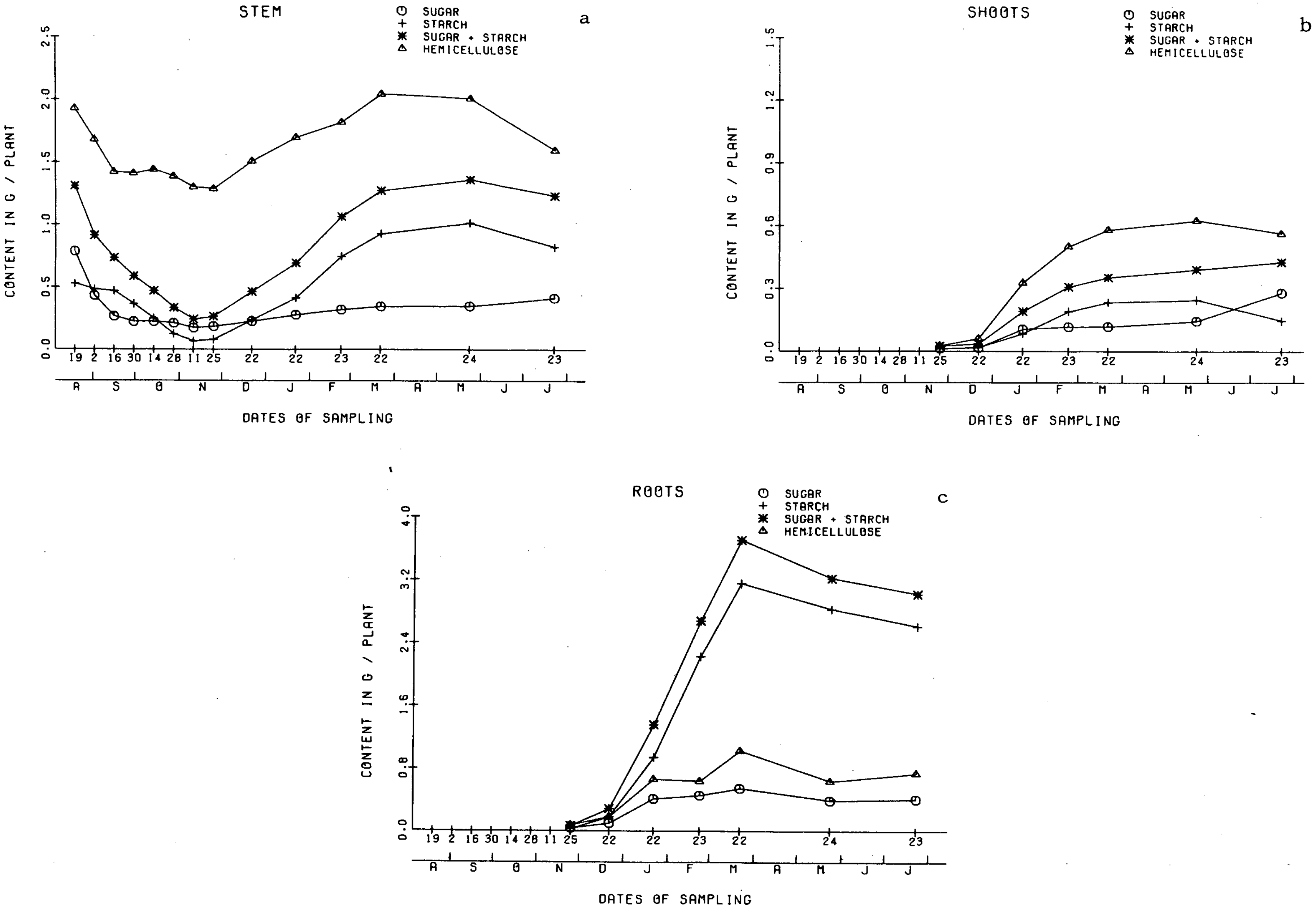


FIG. 34 - Seasonal changes in sugar, starch and hemicellulose content in the stem (a) shoots (b) and roots (c) of Jacquez plants

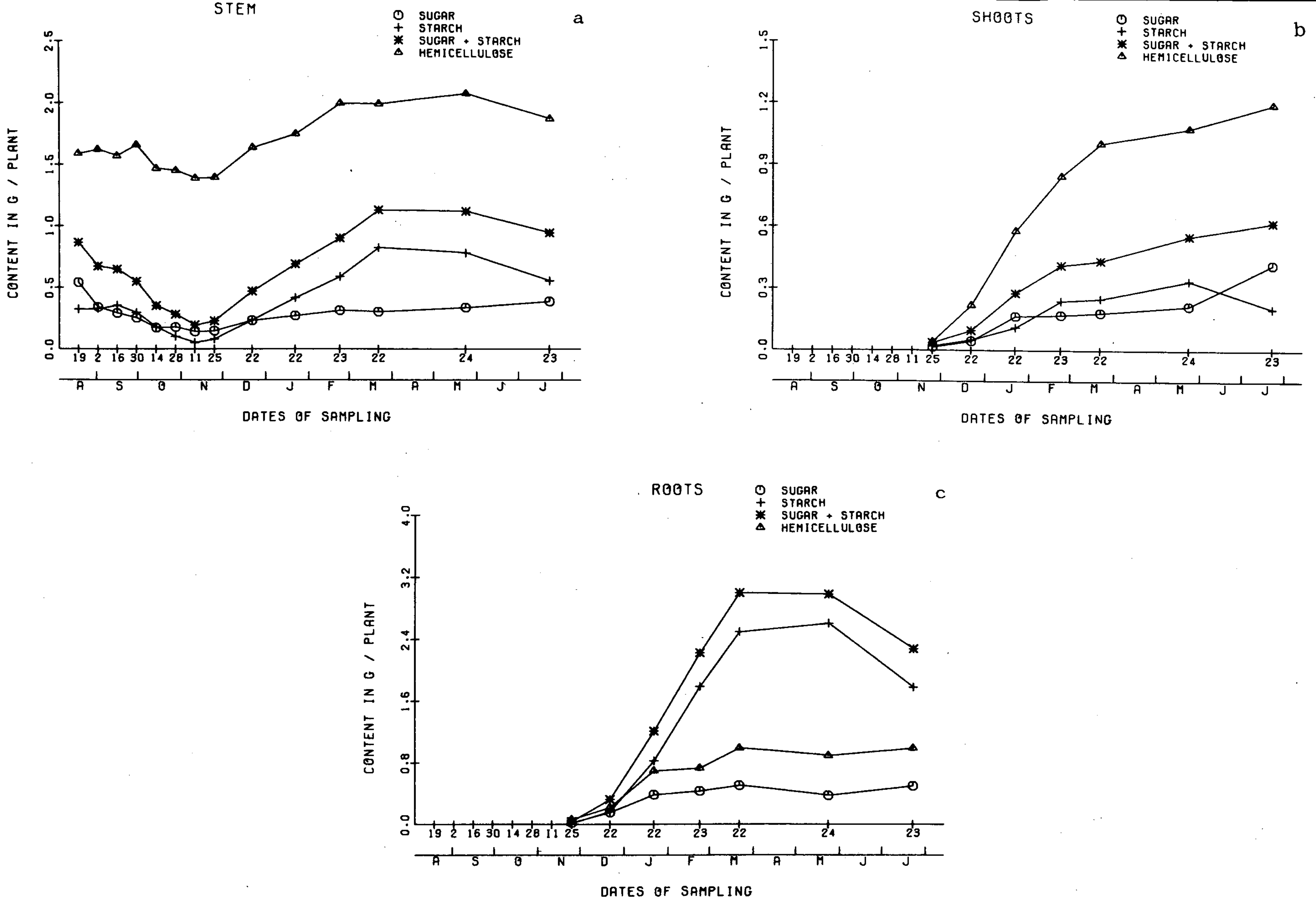


FIG. 35 - Seasonal changes in sugar, starch and hemicellulose content in the stem (a) shoots (b) and roots (c) of Salt Creek plants

SECTION 11

EFFECT OF GROWTH PERIOD AND IBA TREATMENT ON THE LEVELS OF CARBOHYDRATES AND NITROGEN FRACTIONS AND OTHER MACRO-ELEMENTS IN DIFFERENT REGIONS OF CUTTINGS (PLANTS)

11.1 Introduction

In order to obtain a better understanding of the metabolism of the plant during propagation, knowledge of the translocation and utilisation of essential substances like carbohydrates, nitrogenous constituents and nutrient elements can be regarded as of importance. Since growth regulator treatments can be used to improve the rooting ability of cuttings, the effect of this treatment on the abovementioned pro= cesses also seemed of sufficient importance to evaluate. This study was there= fore aimed at gathering information on these aspects.

11.2 Procedure

Cuttings for rooting of Jacquez and Salt Creek were collected at random at the be= ginning of August 1976 and held at $0^{o}C$ until the experiment was commenced on 28 October. This late planting date was chosen because the experiment had to be carried out in the open. Warmer weather at this stage ensured that the metabolic changes could be followed over a comparatively short period of time. The daily minimum, maximum and average temperatures in a Stevenson shelter during the experimental period are presented in Fig. 36.

Because of limitations imposed by the amount of material and facilities available a formal statistical design could not be followed. On advice from a biometrican, an alternative design and statistical analysis was adopted, the experimental procedure being as follows.

On 28 October cuttings of untreated Jacquez and Salt Creek were taken for analysis. On the same day cuttings of four treatments were planted after their initial mass had been determined. The treatments were as follows.

Jacquez control

Jacquez treated with 1 000 mg/1 IBA

Salt Creek control

Salt Creek treated with 1 000 mg/1 IBA

Sampling dates for the above treatments were 4, 11, 25 November and 9, 23 Decem= ber. Three replicates of 40 cuttings each were used for each sampling date / treat= ment combination. IBA treatments were applied by dipping the freshly cut basal end of the cuttings 1 cm deep in the solution for 5 minutes.

The cuttings were planted in acid washed sand according to the procedure described in section 3.5 and watered only with deionised water. When sampling, the plants were removed from the medium according to the procedure described in section 3.5. After washing with deionised water, the plants were divided into four regions as shown in Fig. 37. Where shoots and roots had developed they were dried and weighed separately, but for analysis again combined with the apical and basal re= gions. The samples were analysed for sugar, starch, hemicellulose, total N, soluble N, P, K, Ca and Mg. Components were expressed as content per plant or per region. These values were corrected to the average initial cutting mass for each cultivar. Some of the data was also expressed as concentration of initial dry mass (Table 14). For the dry mass results the plants were divided into shoots, stem and roots.

11.3 Results and discussion

11.3.1 Dry mass changes

The effect of growth period and hormone treatment on the whole plant, stem, shoot and root dry mass is presented in Fig. 38 and Appendix 34.

The dry mass of the whole plant decreased over the full growing period, reaching the lowest values after 56 days. Losses from initial dry mass varied between 7,6 and 10,6%. IBA treatment had no significant effect on dry mass loss in the whole plant. Buttrose (1966), working on Sultana cuttings under warmer con= ditions, recorded a maximum dry mass loss after 50 days' growth. These losses were slightly higher than in the present study.

Dry mass of the stem decreased between 12,2 and 14,2% over the 56-day period. This is somewhat lower than the maximum loss of 20-22% recorded by Buttrose (1966).

The buds had already developed sufficiently after 14 days to enable them to be broken from the stem. Compared to this, the first roots were just visible in the IBA-treated Salt Creek after 28 days. The dry mass of the shoots was always considerably greater than that of the roots. There was no significant effect of

IBA treatment on shoot growth. On the other hand, root growth in both cultivars was stimulated by IBA treatment.

11.3.2 Whole plant changes

11.3.2.1 Sugar, starch and hemicellulose

The effects of growth period and hormone treatment on sugar, starch and hemi= cellulose content in whole Jacquez and Salt Creek plants are presented in Figs. 39a, 40a, 41a and 42a and Table 8.1. The changes over the full period as well as the maximum loss from the initial content in mg; and as a percentage, are also pre= sented in Table 8.1.

In both cultivars the initial starch content was higher than that of the initial sugar content. Because of the initially high starch content, it showed the largest per= centage loss over the full period. With the exception of the IBA-treated Salt Creek which showed a small but non-significant starch increase over the first 7 days, starch decreased constantly to reach the lowest content after 56 days.

Sugar content in both Jacquez treatments and the Salt Creek control showed the largest decrease during the first 14 days. The IBA-treated Salt Creek showed the largest decrease in sugar content during the first 7 days.

Although the results of this study were expressed as content and those of Buttrose (1966) as concentration of initial dry mass, they are comparable in certain respects.

These studies showed the following similarities with regard to sugar and starch.

. Initially higher starch than sugar values.

. A small initial loss in sugar followed by reasonably constant sugar values.

. Of the two fractions, starch contributed most to the carbohydrate needs of the developing cutting, resulting in the lowest starch levels being less than those of the corresponding sugar levels.

Hemicellulose in the Jacquez control decreased during the first 7 days, increased over the next 7 days, thereafter decreasing to a minimum after 42 days. Hemi= cellulose content in the other treatments however, showed an initial increase during the first 7 days, and a subsequent loss up to 42 days.

It is clear that some of the hemicellulose in the cutting served as a reserve car= bohydrate. The maximum hemicellulose loss varied between 8, 6% and 11, 5% in Jacquez and 8, 5% and 9, 8% in Salt Creek. These losses were considerably lower than that recorded in two-node cuttings of Sultana (Buttrose, 1966) where 25, 2% of the initial concentration was lost. During the last 14-day period, hemicellulose increased significantly in both Salt Creek treatments, indicating the development of new cell walls.

Both Jacquez treatments showed a steady decrease in sugar + starch + hemicellulose over the full growing period. The Salt Creek treatments reached a minimum content after 42 days.

After 42 days the IBA-treated Salt Creek plants had used slightly more sugar + starch + hemicellulose than the control plants. In Jacquez there was no difference between the two treatments at any stage with regard to sugar + starch + hemicel= lulose.

The treatments of Jacquez showed a considerably larger use of sugar + starch + hemicellulose than the Salt Creek treatments. This is contradictory to the results obtained in the callusing experiment (section 6) where Salt Creek had used more carbohydrates than Jacquez. A possible explanation for this phenomenon could be found in the temperature at which the experiments were carried out: Should the rate of carbohydrate utilisation in Salt Creek due to respiration be more heat-sensitive than that of Jacquez, the higher temperature during callusing (25°C) compared to that during rooting (average 17,6°C air-temperature) might explain the difference. In the study on carbohydrate utilisation at different temperatures (section 8) Salt Creek used considerably less than Jacquez at 15°C whereas at 25°C there was little difference. This result lends substance to the above-mentioned hyphothesis.

Apart from that already mentioned, IBA treatment did not alter the utilisation of carbohydrate fractions in the whole plants to any marked

The possible reasons for carbohydrate losses incurred during this experiment are the same as those described in section 6. The loss of plant parts probably played a larger role in this instance as leaves and roots could be lost during growth, removal of the plants from the growing medium, and preparation of the samples for analysis.

11.3.2.2 Total N and soluble N

The effects of growth period and IBA treatment on total N and soluble N content in Jacquez and Salt Creek plants are presented in Figs. 39b, 40b, 41b, 42b and Table 8.2. The changes over the full period, as well as the maximum loss from the initial content in mg and as a percentage, are also presented in Table 8.2.

Jacquez showed statistically significant losses in total N over the full period. Salt Creek lost less total N in content and as percentage than Jacquez and these losses were not significant. As the cuttings were grown without nutrients, no N could be absorbed, and the loss therefore represents the total loss by the plant. This loss can be ascribed to the following factors. Firstly, leaching from the cutting by the irrigation water. Secondly, a loss of small plant parts such as leaves and roots during growth, removal of plants from the growing medium, and pre= paration of the samples for analysis. On the whole, IBA treatment had no effect on changes in total N content.

Soluble N in Jacquez showed very little change over the full period. The initial increase found in the callusing cuttings (section 6) had probably already taken place in cold storage before this experiment was commenced. The Salt Creek control showed a significant increase in soluble N after 7 days and 56 days, but on the whole there was little change. IBA treatment also had little effect on the soluble N content.

Soluble N comprised approximately 20% of the total N in the plants over the whole period. Compared to these results, Obbink et al. (1973) recorded an initial soluble N : total N ratio of roughly 1:2 in Sultana cuttings. After ten weeks of growth this ratio had changed to roughly 1:6, nearly the same as in the present study. The total N decrease recorded by Obbink et al. (1973) was 3,5% over the 10-week period compared to between 10,5% and 16,9% maximum loss in the present study.

11.3.2.3 Phosphorus, potassium, calcium and magnesium

The effects of growth period and IBA treatment on the content of P, K, Ca and Mg in Jacquez and Salt Creek plants are presented in Figs. 39c, 40c, 41c, 42c and Table 8.3. The changes over the full period as well as the maximum loss from the initial value are also presented in mg and as a per= centage in Table 8.3.

All four elements showed a loss over the full period in all treatments. With the exception of the K in Jacquez control and Mg in both Salt Creek treatments, all losses over the full period were statistically significant. These losses can be ascribed to the same causes as the total N losses. The extent of the losses varied between cultivars. Jacquez showed a larger Ca loss but smaller K and Mg losses than Salt Creek. P proved an exception with the Jacquez con= trol losing less than the other treatments. Apart from this, IBA treatment had one further noticeable effect, namely the samller loss in Mg compared to the control in Jacquez.

11.3.3 Changes per region

11.3.3.1 Sugar, starch and hemicellulose

The effects of growth period and IBA treatment on sugar, starch and hemicellulose content in four regions of Jacquez and Salt Creek plants, are presented in Figs. 43 - 46, 47a, b; 48a, b; 49a, b; 50a, b and Tables 9 - 13.

Sugar + starch content in the Jacquez control plants showed a reasonably linear decrease from planting until 56 days thereafter. The percentage decreases in all regions were more or less the same. Considering the two components separately, the full growing period can be divided into four 14 - day periods. During the first period sugar contributed more to the total loss than starch. During the next 14 days both components contributed about the same amount to the loss. The third period showes a sugar increase and a loss in starch. The fourth period was si= milar to the second, with both sugar and starch losses. The air temperature data (Fig. 36) was investigated in an attempt to provide an explanation for the sugar increase during the third period and the loss during the fourth period, bud did not reveal any possible relationship.

The IBA - treated Jacquez showed basically the same pattern as the control in respect of sugar and starch changes. However, the BR of the IBA treatment had considerably more sugar and less starch after 56 days. IBA treatment there= fore stimulated starch breakdown in the region up to 4 cm from the application site during the last 14 days. This should be seen as a probable indirect effect caused by the more intense root development which had, in turn, been stimulated by the IBA treatment.

Hemicellulose in the Jacquez control plants showed a reasonably constant decrease in the SBR and BR over the 56-day period, indicating that some hemicellulose served as a reserve carbohydrate. The AR showed no significant change over the same period. Hemicellulose in the SAR decreased during the first 42 days, followed by an increase during the last 14 days. This increase might suggest the formation of new cell walls in this area.

Hemicellulose changes in the IBA - treated cuttings differed somewhat from those in the control. Firstly, the SAR and SBR recorded an increase during the first 7 days. Secondly, this was followed by a larger loss than that shown by the control over the next 21-day period. Thirdly, the SBR showed an increase during the last 28 days suggesting cell-wall formation in this region.

Sugar + starch content in the Salt Creek control plants over the full growing period differed somewhat between regions. The AR and BR showed steady losses over the full period. The SAR and SBR showed very small losses during the first 7 days and considerable losses during the next 21 days, followed by increases over the next 14 days. After the full period the different regions had lost about the same percentage of their initial content. The sugar + starch losses could mainly be accounted for by a loss in starch, with the BR showing the largest percentage loss over the full period. Sugar decreased in all parts over the first 28 days, followed by increases up to 56 days.

The IBA - treated Salt Creek showed the same basic pattern of changes in sugar and starch as the control, except for two noteworthy differences. Firstly, IBA delayed starch breakdown in the AR and BR and stimulated sugar - to - starch conversion in the SAR and SBR during the first 7 days. Secondly, the BR of the IBA - treated plants had a larger percentage of the sugar in the plant compared to the control after 56 days. This is most probably a secondary effect caused by the development of roots stimulated by IBA treatment.

Hemicellulose changes in the Salt Creek control and IBA - treated cuttings were very similar. Some hemicellulose was used as a reserve, especially in the SAR and BR over the first 42 days. During the last 14 - day period there was a slight increase in hemicellulose in the SAR and SBR, suggesting the formation of new cell walls in these regions.

The sugar and sugar + starch + hemicellulose as percentage of initial dry mass after 14 and 28 days' growth of Jacquez and Salt Creek control and IBA treated cuttings are presented in Table 14. The significance of these figures will be discussed in section 12.

11.3.3.2 Total N, soluble N, phosphorus, potassium, calcium and magnesium

The effects of growth period and IBA treatment on total and soluble N, P, K, Ca, and Mg content in four regions of Jacquez and Salt Creek plants are presented in Figs. 47c, d; 48c, d; 49c, d; 50c, d; 51 - 54 and Tables 15 - 20.

Three of the elements, N, P and K, showed mobility with redistribution mainly to the AR. Ca and Mg showed less mobility but there was also a tendency for redistribution to the AR. IBA treatment of Jacquez had little effect on trans= location of the mobile elements when compared to the control. Treating Salt Creek with IBA however, stimulated the redistribution of mobile elements to the AR. These are valid examples of a sink/source relationship. In the control plants the AR formed the sink and the other regions the source. IBA treatment of Salt Creek altered the relationship, with the BR also forming a sink for N, P and K. This sink was less powerful than that of the AR.

The results obtained at the end of 56 days' growth compare reasonably well with those recorded by Hosoi et al. (1971) during the first 60 days' growth. At these stages root and shoot growth in both experiments had proceeded to about the same extent. Hosoi et al. (1971) also recorded a higher mobility of N, P and K with considerable translocation to the shoots. Mg showed more mobility, also to the shoots, compared to the results obtained in the present study. Ca was less mobile, as was also found in the present study.

Changes in soluble N were more complicated. The Jacquez control plants showed a decrease in soluble N in the SAR, SBR and BR during the first 7 days, while the AR showed no change. During the following 21 days there was an increase in all regions. From 28 to 56 days all regions showed an overall loss. The fact that the AR lost soluble N during the period of most active shoot growth, while the total N increased, shows the extent to which soluble N is transformed to insoluble N once it has been translocated to the AR.

The most important difference between the control and IBA - treated Jacquez with regard to soluble N, was the increase in the latter over the first 7 days. This effect was significant in the SBR.

The Salt Creek control behaved similarly to the IBA-treated Jacquez, showing increases in soluble N in al regions during the first 7 days followed by losses up to 14 days. It subsequently increased slightly in all regions for the rest of the growth period.

The most important effect of IBA treatment on the soluble N content of Salt Creek was the large increase in the BR over the whole period. This increase accompanied the stimulated root development. Comparison of the increase in soluble N to that of total N in this region, shows that the former is larger than the latter. This means that most of the soluble N originated in this region.

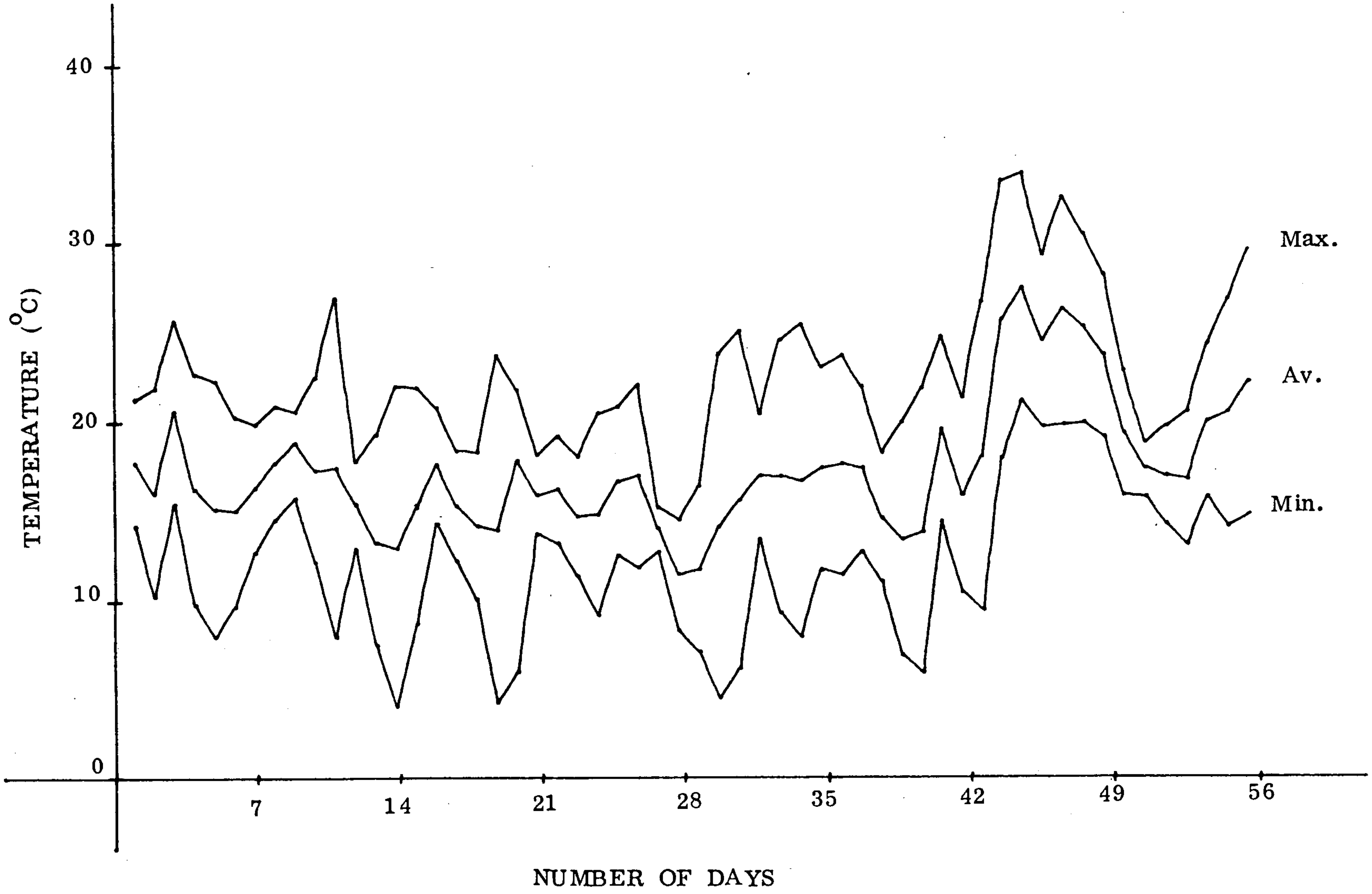


FIG. 36- Daily temperatures in the shade during growth of Jacquez and Salt Creek plants from cuttings (28 October - 23 December 1976)

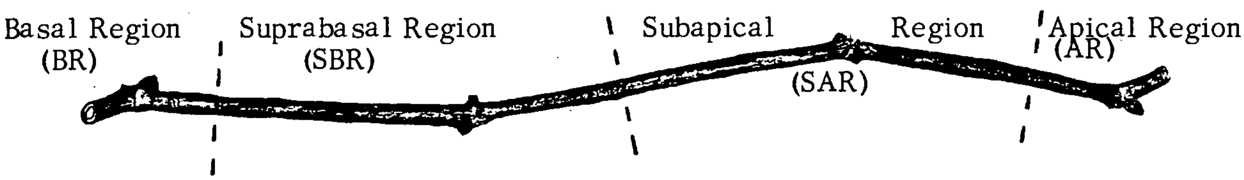


FIG. 37 - Division of cuttings (plants) into four regions for sampling

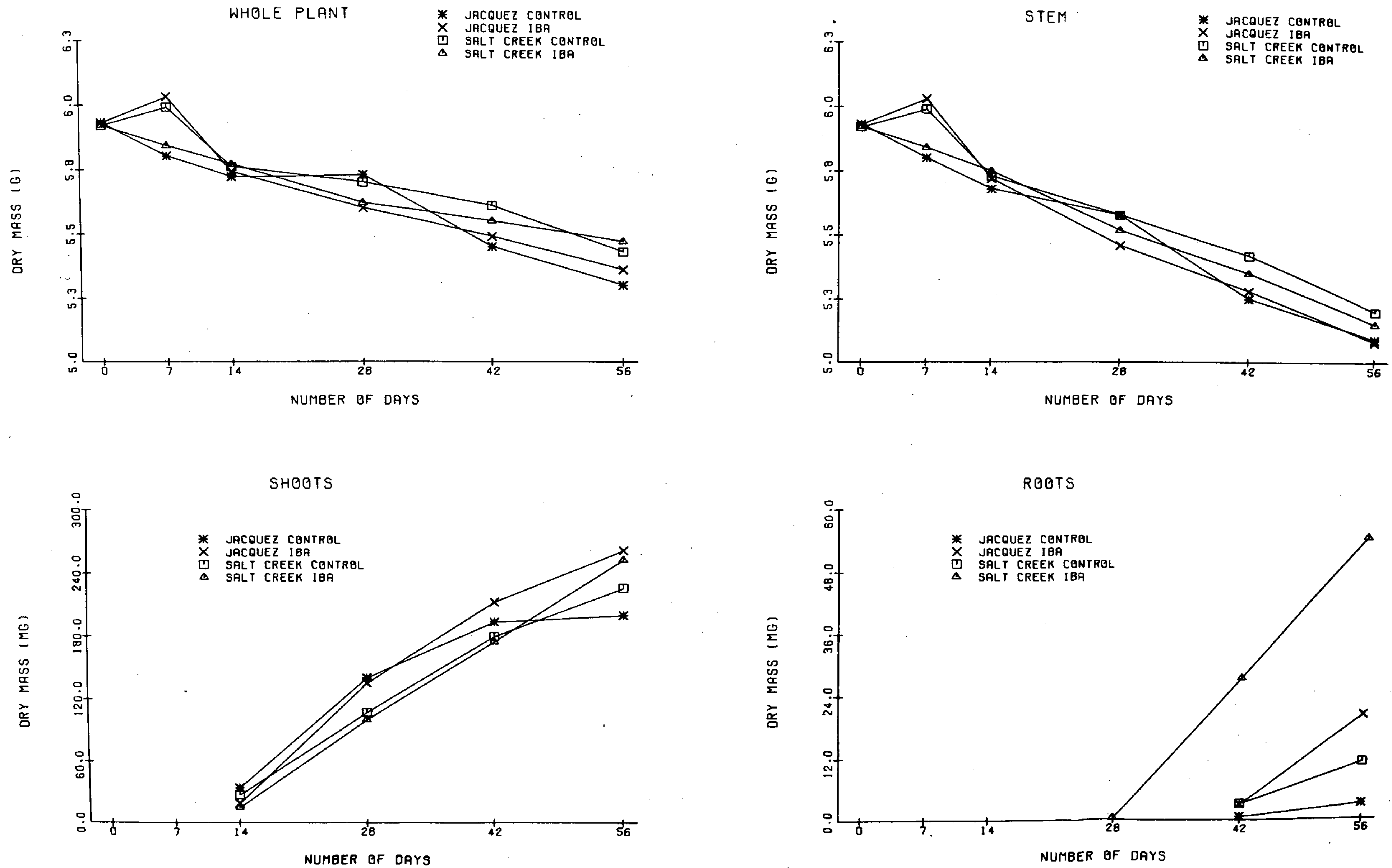


FIG. 38 - Effect of growth period and IBA treatment on dry mass of the whole plant (a), stem (b), shoots (c) and roots (d) of Jacquez and Salt Creek

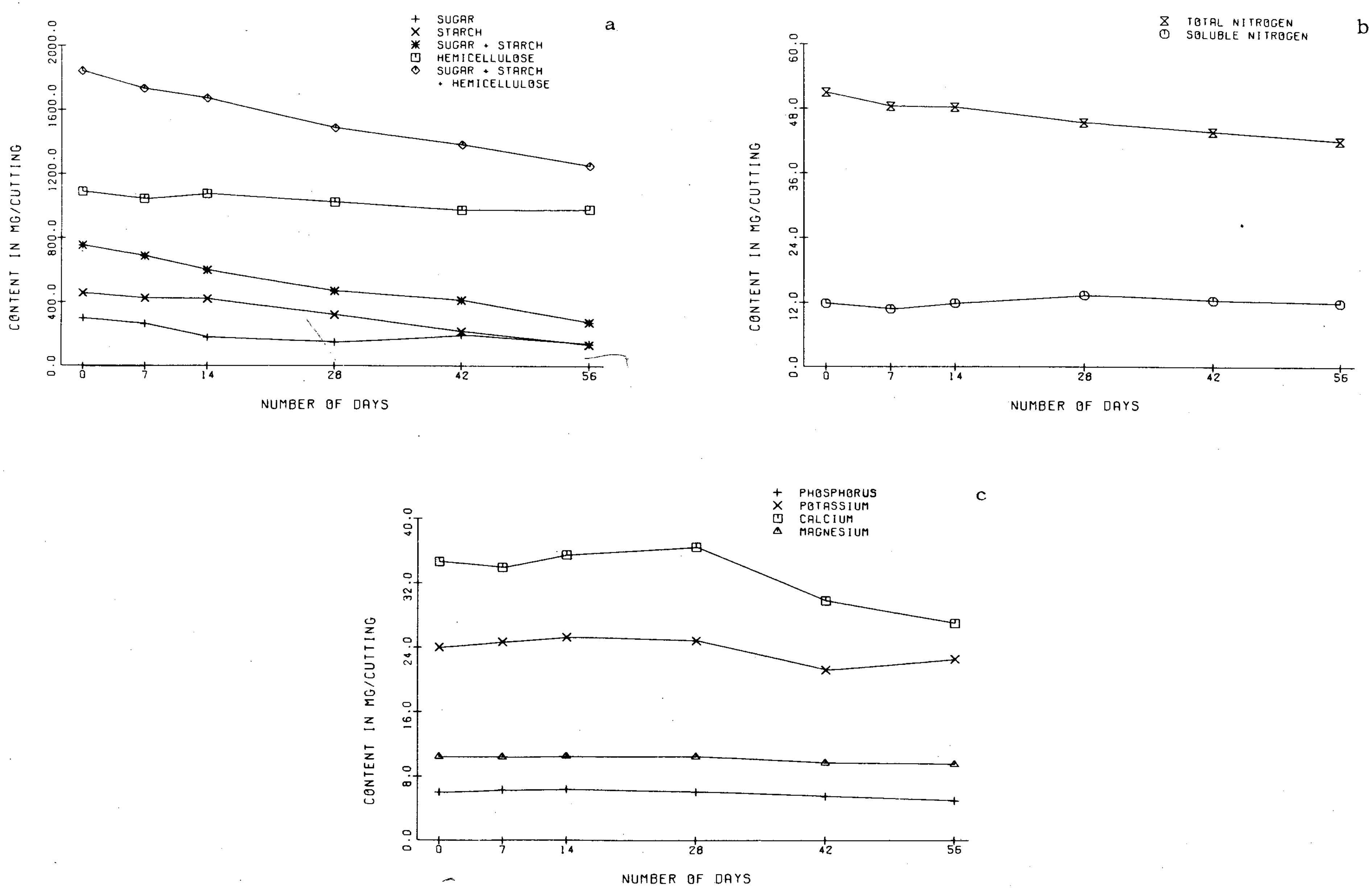


FIG. 39 - Effect of growth period on sugar, starch and hemicellulose (a), total N and soluble N (b), phosphorus, potassium, calcium and magnesium (c) content in Jacquez control plants

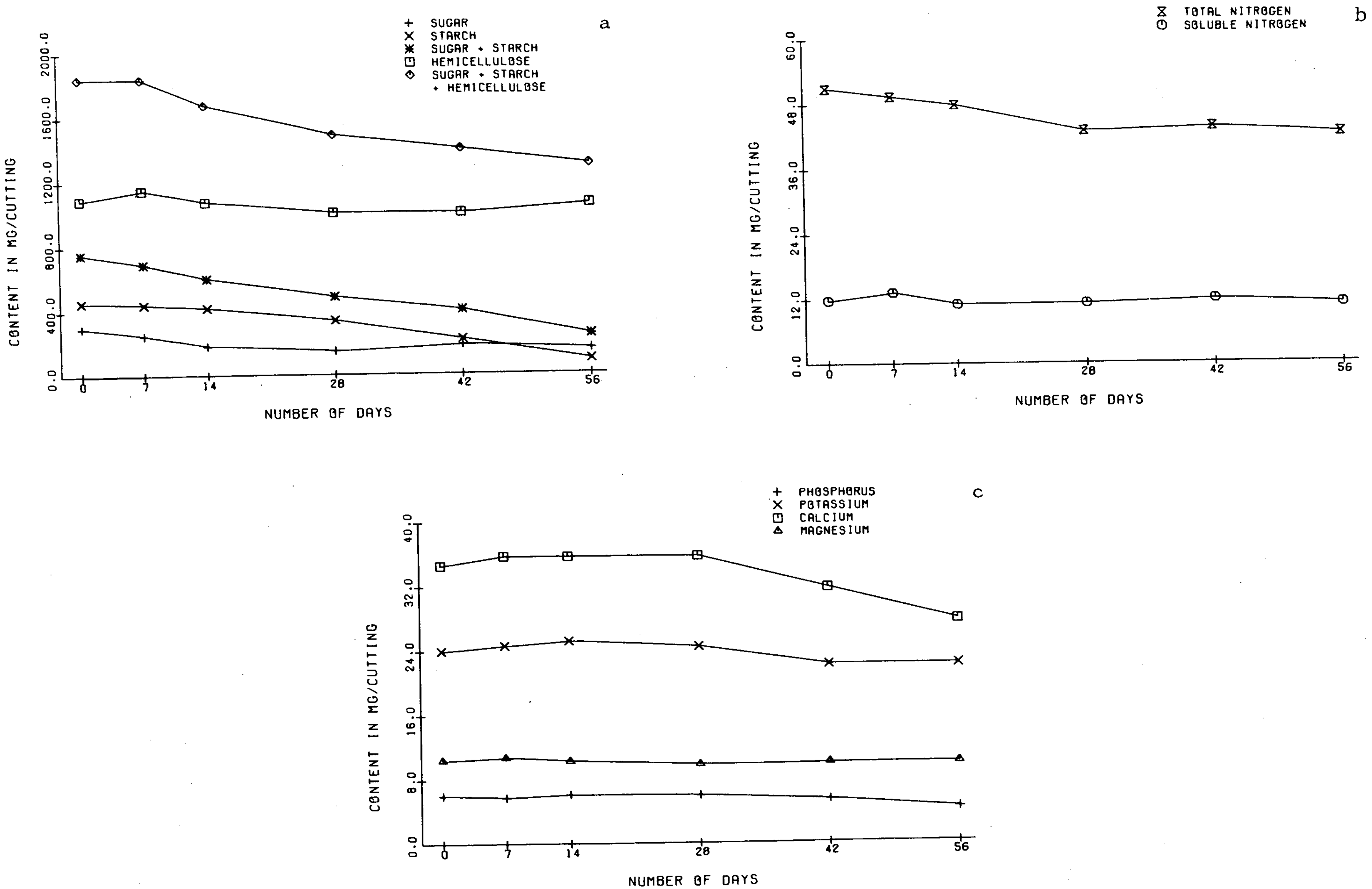


FIG. 40 - Effect of growth period on sugar, starch and hemicellulose (a), total N and soluble N (b), phosphorus, potassium, calcium and magnesium (c) content in Jacquez plants treated with IBA

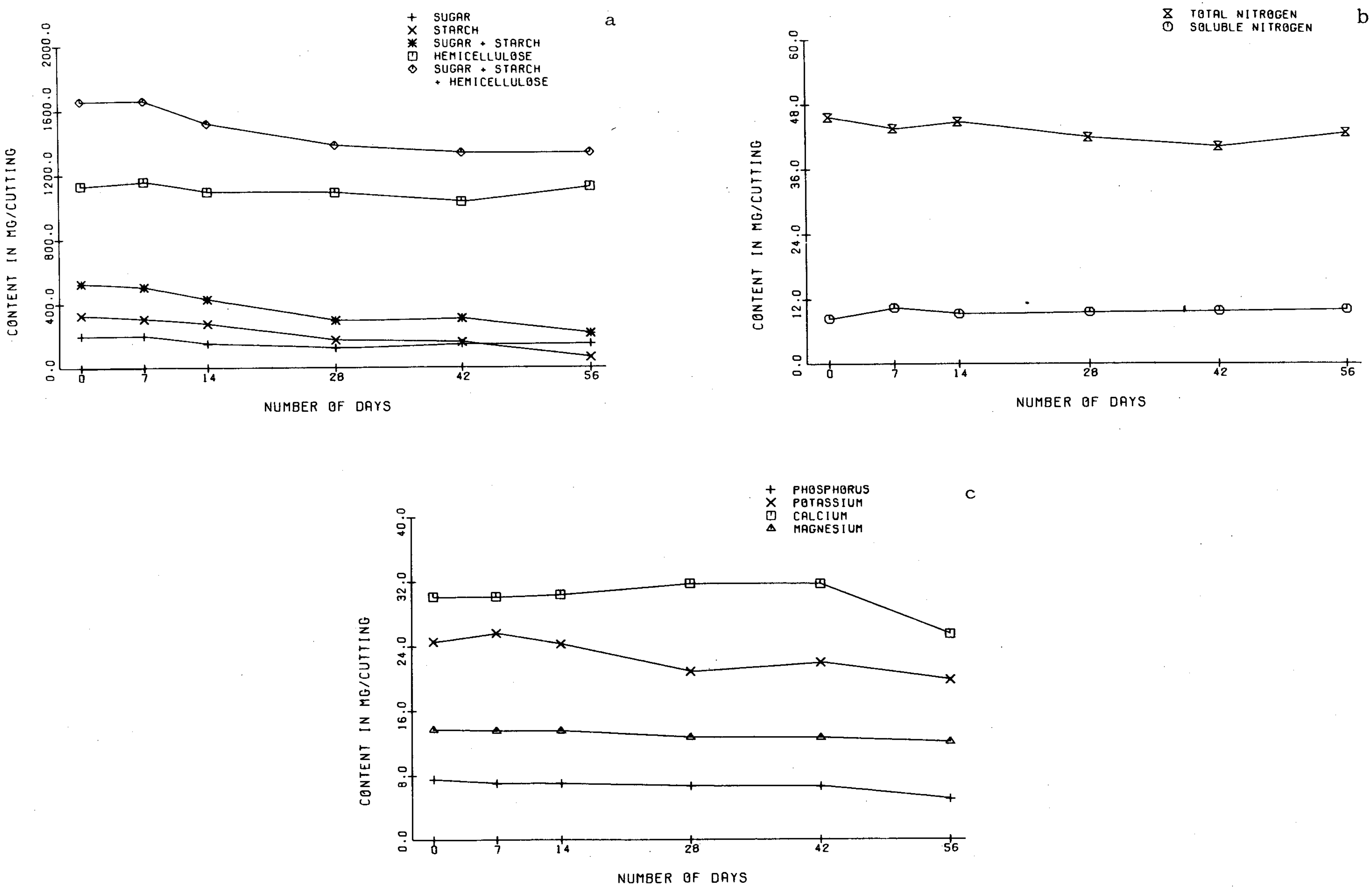


FIG. 41 - Effect of growth period on sugar, starch and hemicellulose (a), total N and soluble N (b), phosphorus, potassium, calcium and magnesium (c) content in Salt Creek control plants

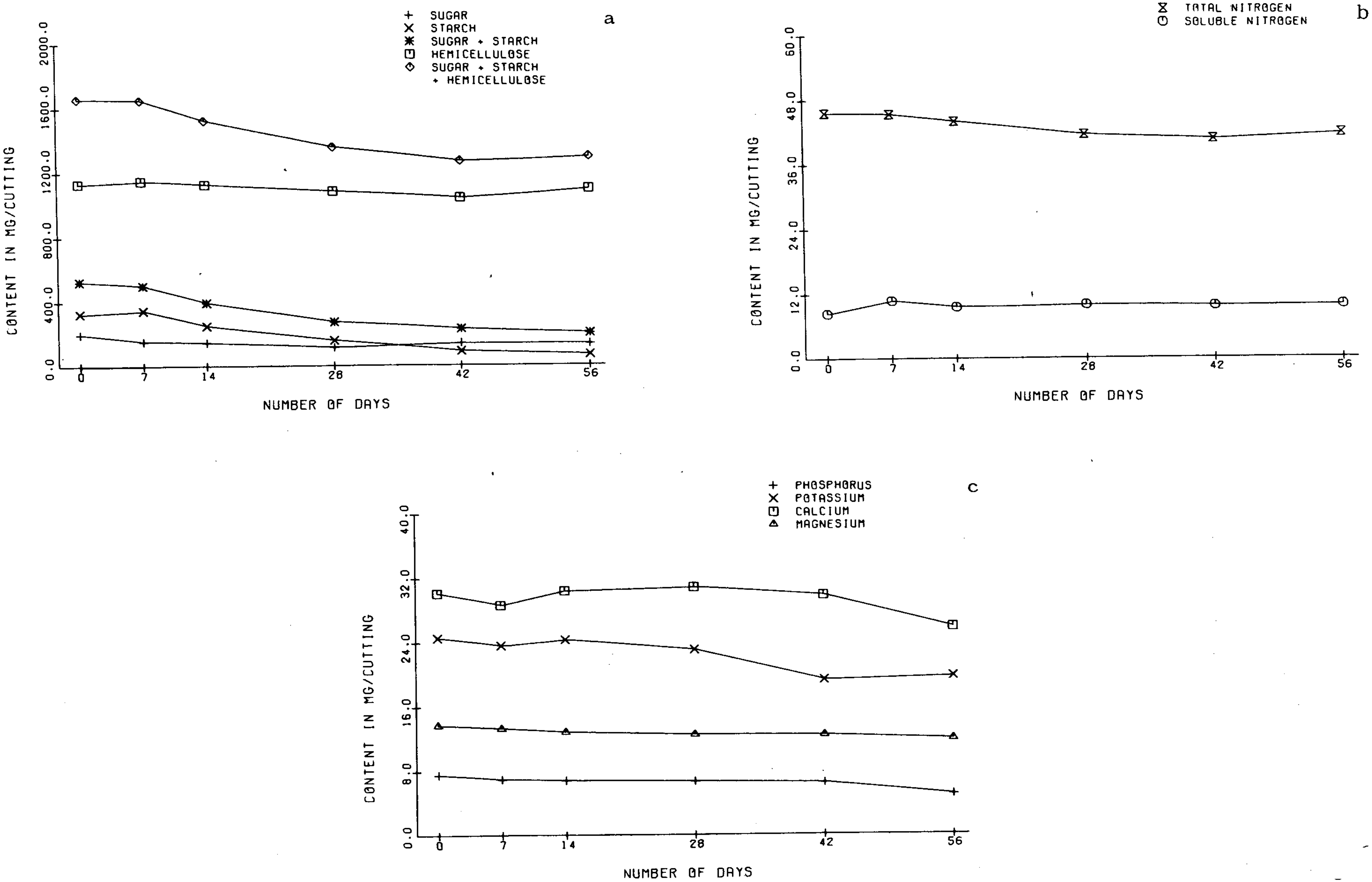


FIG. 42 - Effect of growth period on sugar, starch and hemicellulose (a), total N and soluble N (b), phosphorus, potassium, calcium and magnesium (c) content in Salt Creek plants treated with IBA

TABLE 8.1 - Effect of growth period and IBA treatment on sugar, starch and hemicellulose content in Jacquez and Salt Creek plants

Component	Rootstock and treatment	Content (mg)						Loss (-) or increase (+)			
		Initially	Growth period (days)					Initial to lowest value		Initial to last value if not the same as lowest value	
			7	14	28	42	56	mg	%	mg	%
Sugar	Jacquez control	297,1 C	a 262,9 C	a 178,6 AB	a 146,5 AB	a 192,3 B	a 138,0 A	-159,1	-53,5	-	-
	Jacquez IBA treated	(297,1) C	a 247,5 C	a 181,0 B	a 146,4 A	a 180,8 B	a 155,2 A	-143,4	-48,3	-141,9	-47,8
	Salt Creek control	198,1 B	b 198,0 B	a 149,7 A	a 122,4 A	a 146,1 A	a 148,1 A	- 75,7	-38,2	- 50,0	-25,2
	Salt Creek IBA treated	(198,1) C	a 153,8 B	a 144,8 B	a 115,1 A	a 138,4 AB	a 133,4 B	- 83,0	-41,9	- 64,7	-32,7
Starch	Jacquez control	455,3 D	a 422,2 D	a 416,9 D	a 315,6 C	a 213,6 B	a 132,5 A	-322,8	-70,9	-	-
	Jacquez IBA treated	(455,3) D	a 440,3 D	a 416,6 D	a 334,6 C	a 215,2 B	a 87,7 A	-367,6	-80,7	-	-
	Salt Creek control	327,2 D	a 302,3 CD	a 272,6 C	a 170,2 B	b 155,4 B	a 63,1 A	-264,1	-80,7	-	-
	Salt Creek IBA treated	(327,2) D	a 346,1 D	a 249,0 C	a 156,7 B	a 88,1 A	a 65,6 A	-261,6	-79,9	-	-
Sugar + starch	Jacquez control	752,4 E	a 685,1 D	a 595,5 C	a 462,1 B	a 405,9 B	a 270,5 A	-481,9	-64,0	-	-
	Jacquez IBA treated	(752,4) F	a 687,8 E	a 597,6 D	a 481,0 C	a 396,0 B	a 242,9 A	-509,5	-67,7	-	-
	Salt Creek control	525,3 D	a 500,3 D	a 422,3 C	a 292,6 B	b 302,5 B	a 211,2 A	-314,1	-59,8	-	-
	Salt Creek IBA treated	(525,3) D	a 499,9 D	a 393,8 C	a 271,8 B	a 226,5 A	a 199,1 A	-326,2	-62,1	-	-
Hemicellulose	Jacquez control	1088,0 D	a 1040,0 BC	a 1069,0 CD	a 1014,0 B	a 963,3 A	a 969,7 A	-124,7	-11,5	-118,3	-10,9
	Jacquez IBA treated	(1088,0) B	b 1145,0 C	a 1070,0 B	a 998,0 A	a 994,7 A	b 1050,0 AB	- 93,3	- 8,6	- 38,0	- 3,5
	Salt Creek control	1131,0 CD	a 1154,0 D	a 1088,0 B	a 1082,0 B	a 1020,0 A	a 1114,0 BC	-111,0	- 9,8	- 38,0	- 1,5
	Salt Creek IBA treated	(1131,0) BC	a 1146,0 C	a 1123,0 BC	a 1080,0 B	a 1035,0 A	a 1093,0 B	- 96,0	- 8,5	- 38,0	- 3,4
Sugar + starch + hemicellulose	Jacquez control	1840,0 F	a 1725,1 E	a 1664,5 D	a 1476,0 C	a 1369,2 B	a 1240,2 A	-600,0	-32,6	-	-
	Jacquez IBA treated	(1840,0) E	b 1832,8 E	a 1667,6 D	a 1479,0 C	a 1390,7 B	a 1293,0 A	-547,0	-29,7	-	-
	Salt Creek control	1657,0 C	a 1654,3 C	a 1510,3 B	a 1374,6 A	b 1322,5 A	a 1325,2 A	-335,0	-20,2	-332,0	-20,0
	Salt Creek IBA treated	(1657,0) D	a 1645,9 D	a 1516,8 C	a 1351,6 B	a 1261,5 A	a 1292,9 AB	-396,0	-23,9	-365,0	-22,0

A - F : Compare in the same horizontal row

a, b : Compare in the same vertical column seperately for each component and cultivar

Means accompanied by a common letter are not different at the 5% level

TABLE 8.2 - Effect of growth period and IBA treatment on total N and soluble N content in Jacquez and Salt Creek plants

Component	Rootstock and treatment	Content (mg)						Loss (-) or increase (+)			
		Initially	Growth period (days)					Maximum		Initial to last value if not the same as maximum	
			7	14	28	42	56	mg	%	mg	%
Total N	Jacquez control	50,9 D	a 48,4 CD	a 48,3 CD	b 45,7 BC	a 44,1 AB	a 42,3 A	-8,6	-16,9	-	-
	Jacquez IBA treated	(50,9) C	a 49,4 BC	a 48,0 B	a 43,2 A	a 43,9 A	a 42,8 A	-8,1	-15,9	-	-
	Salt Creek control	45,6 B	a 43,5 AB	a 44,8 AB	a 42,0 AB	a 40,3 A	a 43,0 AB	-5,3	-11,6	-2,6	-5,7
	Salt Creek IBA treated	(45,6) A	a 45,4 A	a 44,1 A	a 41,6 A	a 40,8 A	a 41,9 A	-4,8	-10,5	-3,7	-8,1
Soluble N	Jacquez control	11,8 A	a 10,8 A	a 11,9 A	b 13,4 A	a 12,4 A	a 11,9 A	+1,6	+13,6	+0,1	+0,9
	Jacquez IBA treated	(11,8) A	b 13,3 B	a 11,1 A	a 11,2 A	a 11,9 A	a 11,10 A	+1,5	+12,7	-	-
	Salt Creek control	8,4 A	a 10,4 B	a 9,3 AB	a 9,6 AB	a 9,8 AB	a 10,1 B	+2,0	+23,8	+1,7	+20,2
	Salt Creek IBA treated	(8,4) A	a 10,8 A	a 9,6 A	a 10,0 A	a 9,7 A	a 9,9 A	+2,4	+28,6	+1,5	+17,9

A - D : Compare in the same horizontal row

a, b : Compare in the same vertical column seperately for each component and cultivar

Means accompanied by a common letter are not different at the 5% level

TABLE 8.3 - Effect of growth period and IBA treatment on phosphorus, potassium, calcium and magnesium content in Jacquez and Salt Creek plants

Component	Rootstock and treatment	Content (mg)						Loss (-) or increase (+)			
		Initially	Growth period (days)					Initial to lowest value		Initial to last value if not the same as lowest value	
			7	14	28	42	56	mg	%	mg	%
Phosphorus	Jacquez control	5,99 B	a 6,28 B	a 6,37 B	a 6,05 B	a 5,52 AB	b 4,95 A	-1,04	-17,4	-	-
	Jacquez IBA treated	(5,99) B	a 5,72 B	a 6,09 B	a 5,93 B	a 5,30 B	a 4,20 A	-1,79	-29,9	-	-
	Salt Creek control	7,52 B	a 7,04 B	a 7,02 B	a 6,73 B	a 6,60 B	a 5,03 A	-2,49	-33,1	-	-
	Salt Creek IBA treated	(7,52) B	a 6,96 B	a 6,83 B	a 6,68 B	a 6,44 B	a 4,96 A	-2,56	-34,0	-	-
Potassium	Jacquez control	24,00 B	a 24,63 B	a 25,27 B	a 24,80 B	a 21,19 A	a 22,52 AB	-2,81	-11,7	-1,48	-6,2
	Jacquez IBA treated	(24,00) A	a 24,66 A	a 25,27 A	a 24,37 A	b 22,01 A	a 21,95 A	-2,05	- 8,5	-	-
	Salt Creek control	24,59 C	b 25,63 C	a 24,27 B	a 20,81 AB	b 21,87 B	a 19,70 A	-4,89	-19,9	-	-
	Salt Creek IBA treated	(24,59) B	a 23,65 B	a 24,32 B	b 22,99 B	a 19,16 A	a 19,57 A	-5,43	-22,1	-5,02	-20,4
Calcium	Jacquez control	34,65 CD	a 33,90 C	a 35,47 CD	a 36,43 D	a 29,76 B	a 26,96 A	-7,69	-22,2	-	-
	Jacquez IBA treated	(34,65) C	b 35,78 C	a 35,72 C	a 35,59 C	a 31,53 B	a 27,43 A	-7,22	-20,8	-	-
	Salt Creek control	30,10 B	a 30,16 B	a 30,42 B	a 31,70 B	b 31,60 B	a 25,34 A	-4,76	-15,8	-	-
	Salt Creek IBA treated	(30,10) B	a 28,66 B	a 30,27 B	a 30,77 B	a 29,67 B	a 25,70 A	-4,40	-14,6	-	-
Magnesium	Jacquez control	10,44 B	a 10,38 B	a 10,54 B	b 10,43 B	a 9,67 A	a 9,47 A	-0,97	- 9,3	-	-
	Jacquez IBA treated	(10,44)BC	a 10,71 C	a 10,27ABC	a 9,74 A	a 9,86 AB	a 9,75 A	-0,70	- 6,7	-0,69	-6,6
	Salt Creek control	13,69 A	a 13,12 A	b 13,53 A	a 12,66 A	a 12,57 A	a 12,01 A	-1,68	-12,3	-	-
	Salt Creek IBA treated	(13,69) A	a 13,28 A	a 12,84 A	a 12,44 A	a 12,31 A	a 11,79 A	-1,90	-13,9	-	-

A - D : Compare in the same horizontal row

a, b : Compare in the same vertical column seperately for each component and cultivar

Means accompanied by a common letter are not different at the 5% level

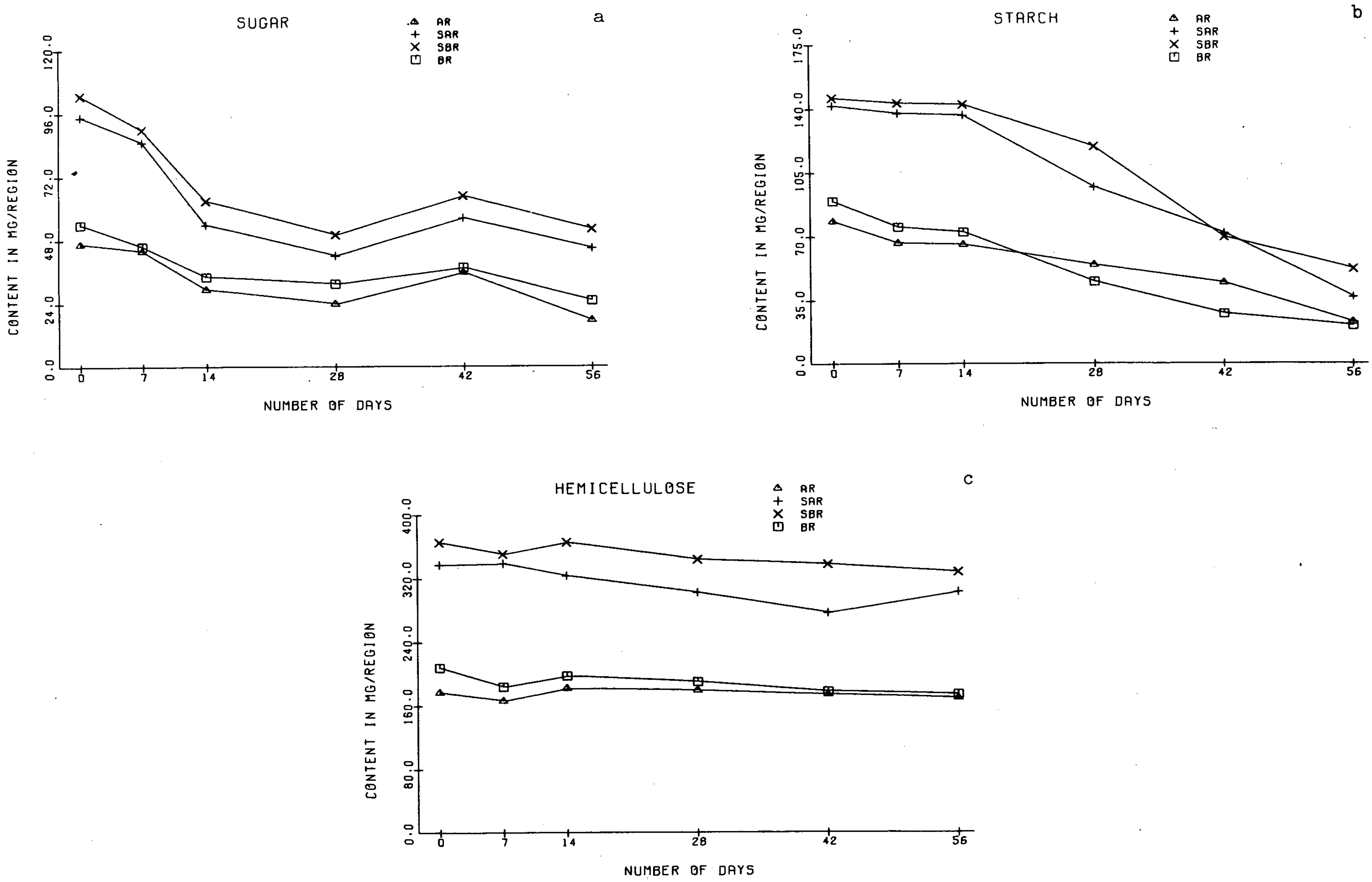


FIG. 43 - Effect of growth period on sugar (a), starch (b), and hemicellulose (c) content in four regions of Jacquez control plants

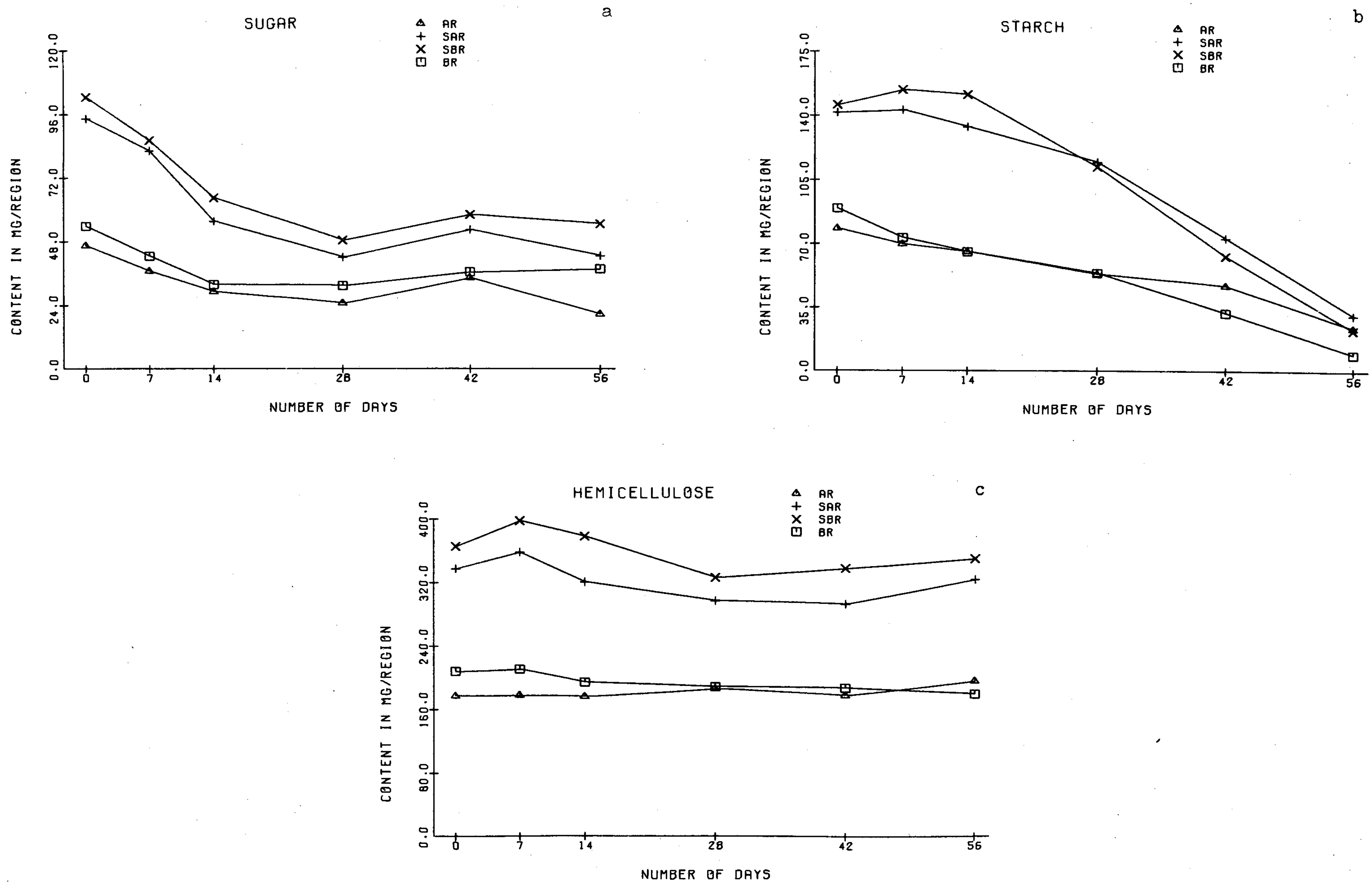


FIG. 44 - Effect of growth period on sugar (a), starch (b), and hemicellulose (c) content in four regions of Jacquez plants treated with IBA

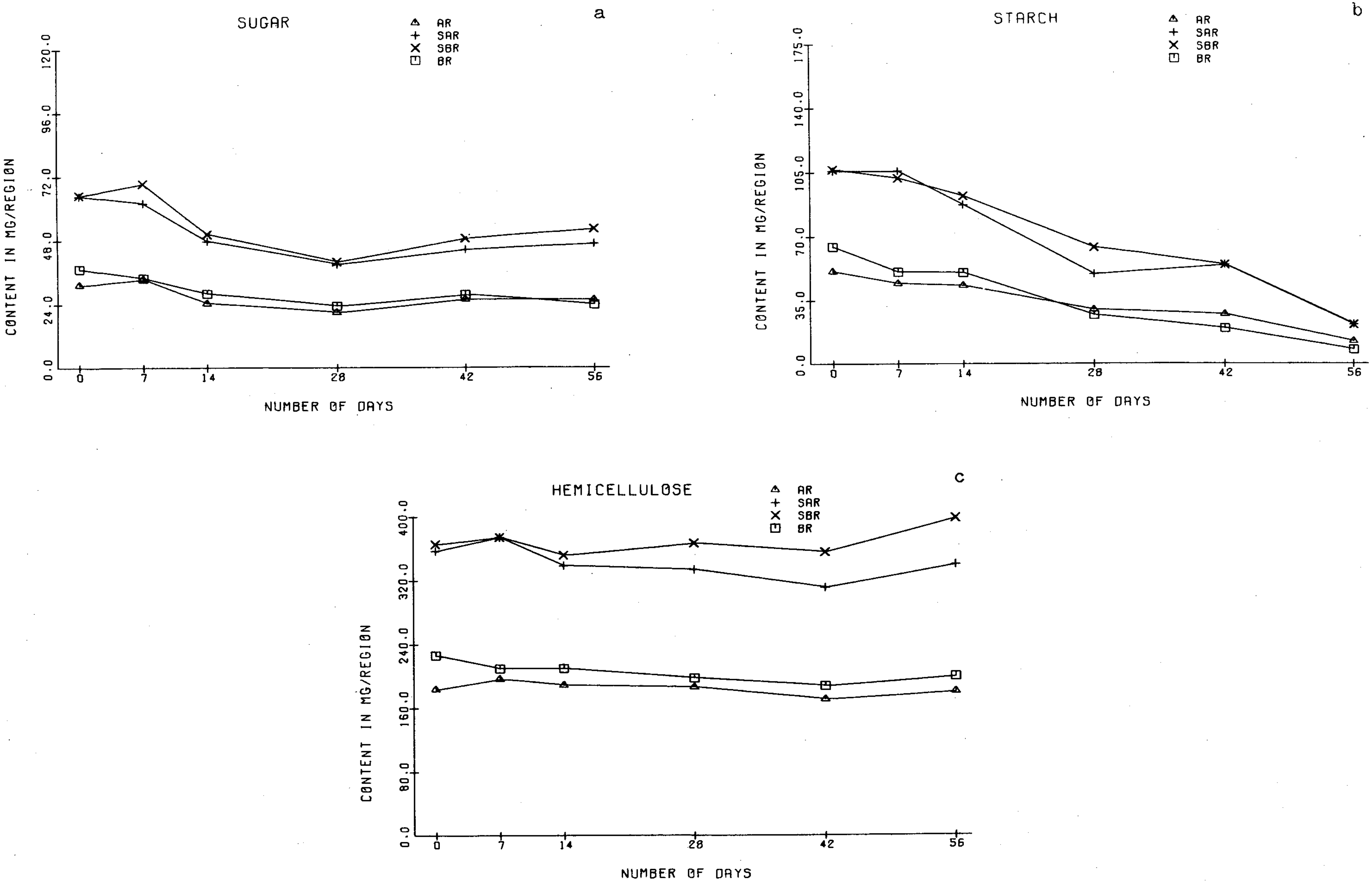


FIG. 45 - Effect of growth period on sugar (a), starch (b) and hemicellulose (c) content in four regions of Salt Creek control plants

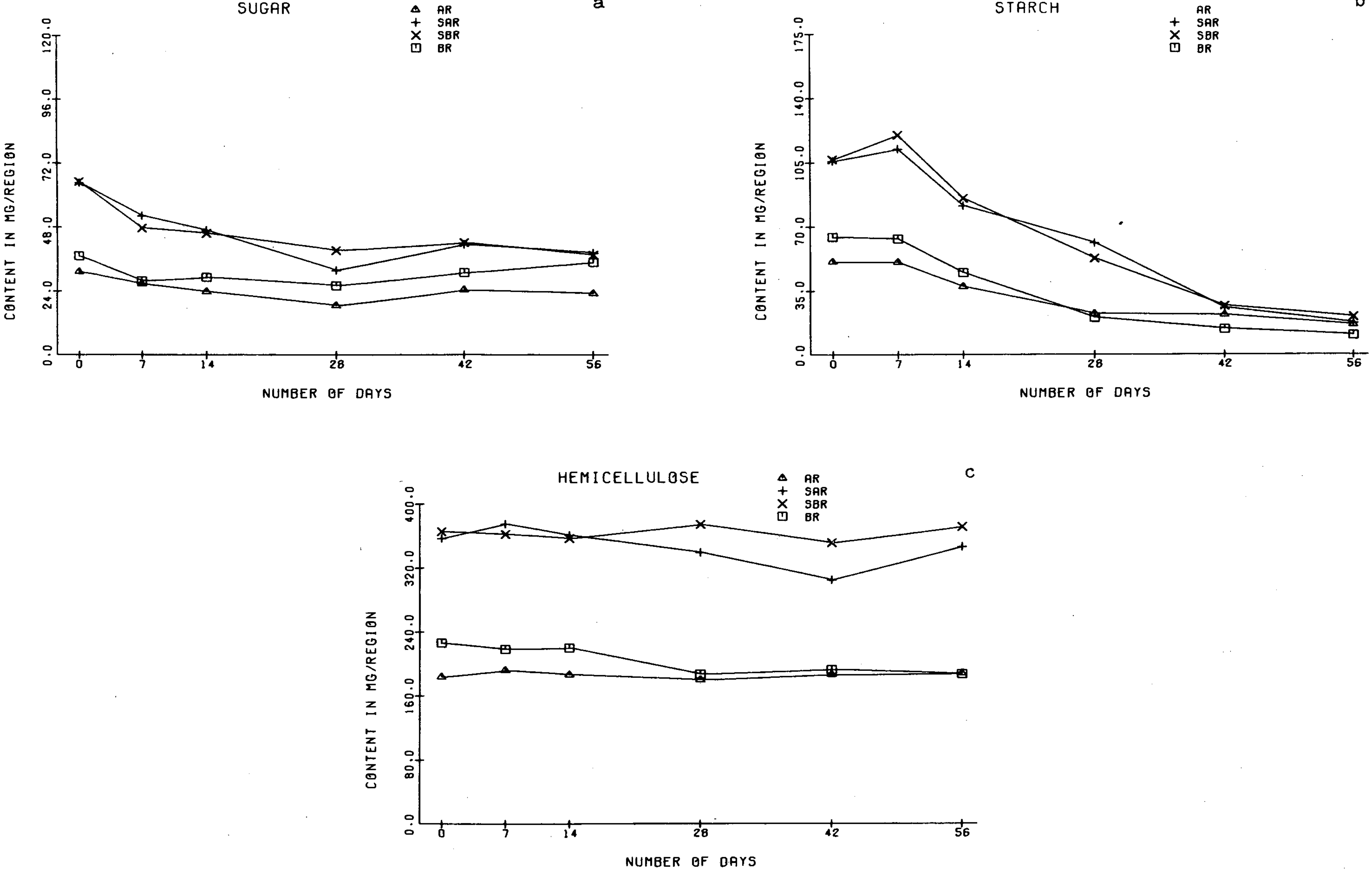


FIG. 46 - Effect of growth period on sugar (a), starch (b), and hemicellulose (c) content in four regions of Salt Creek plants treated with IBA

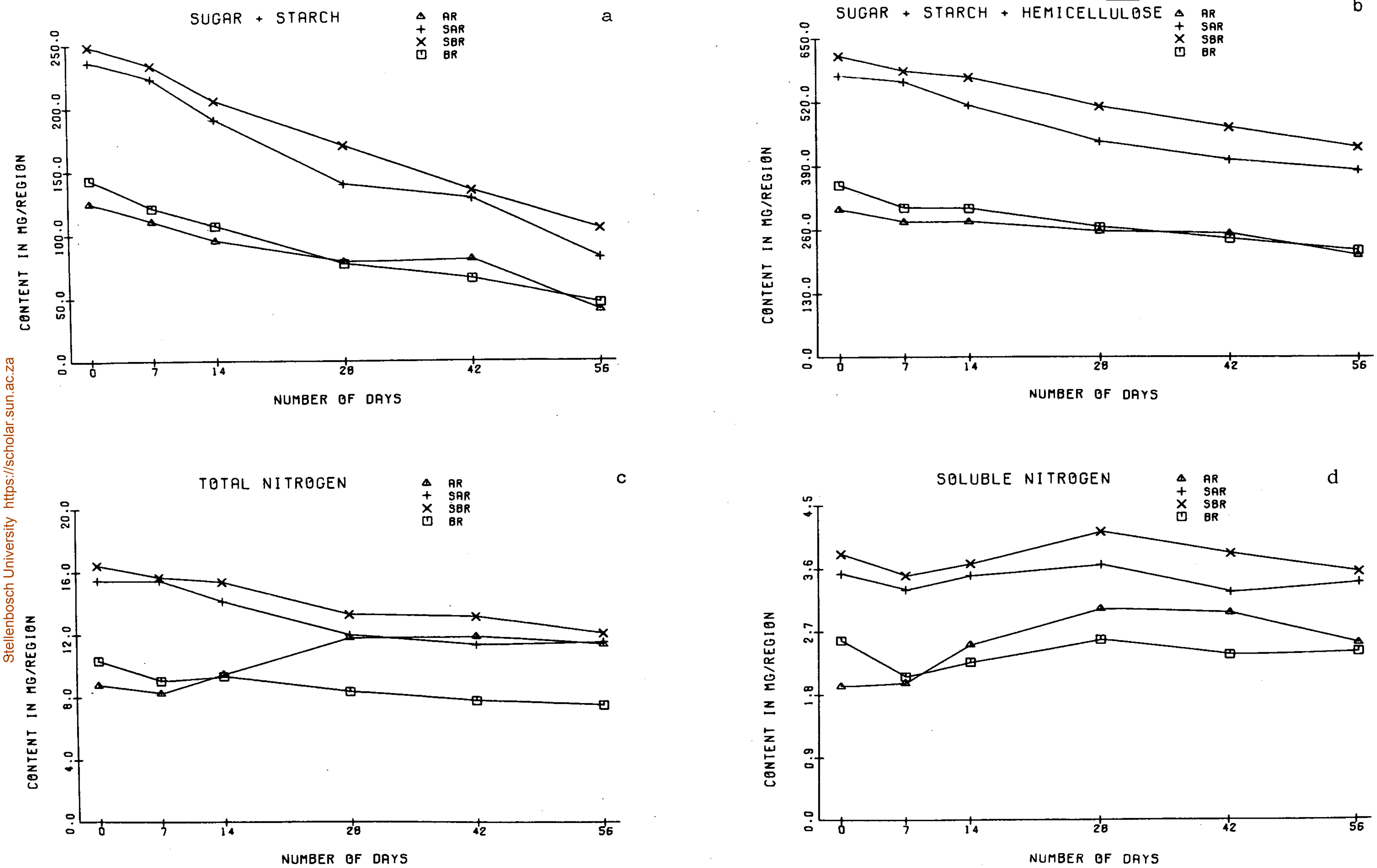


FIG. 47 - Effect of growth period on sugar + starch (a), sugar + starch + hemicellulose (b), total N (c), and soluble N (d) content in four regions of Jacquez control plants

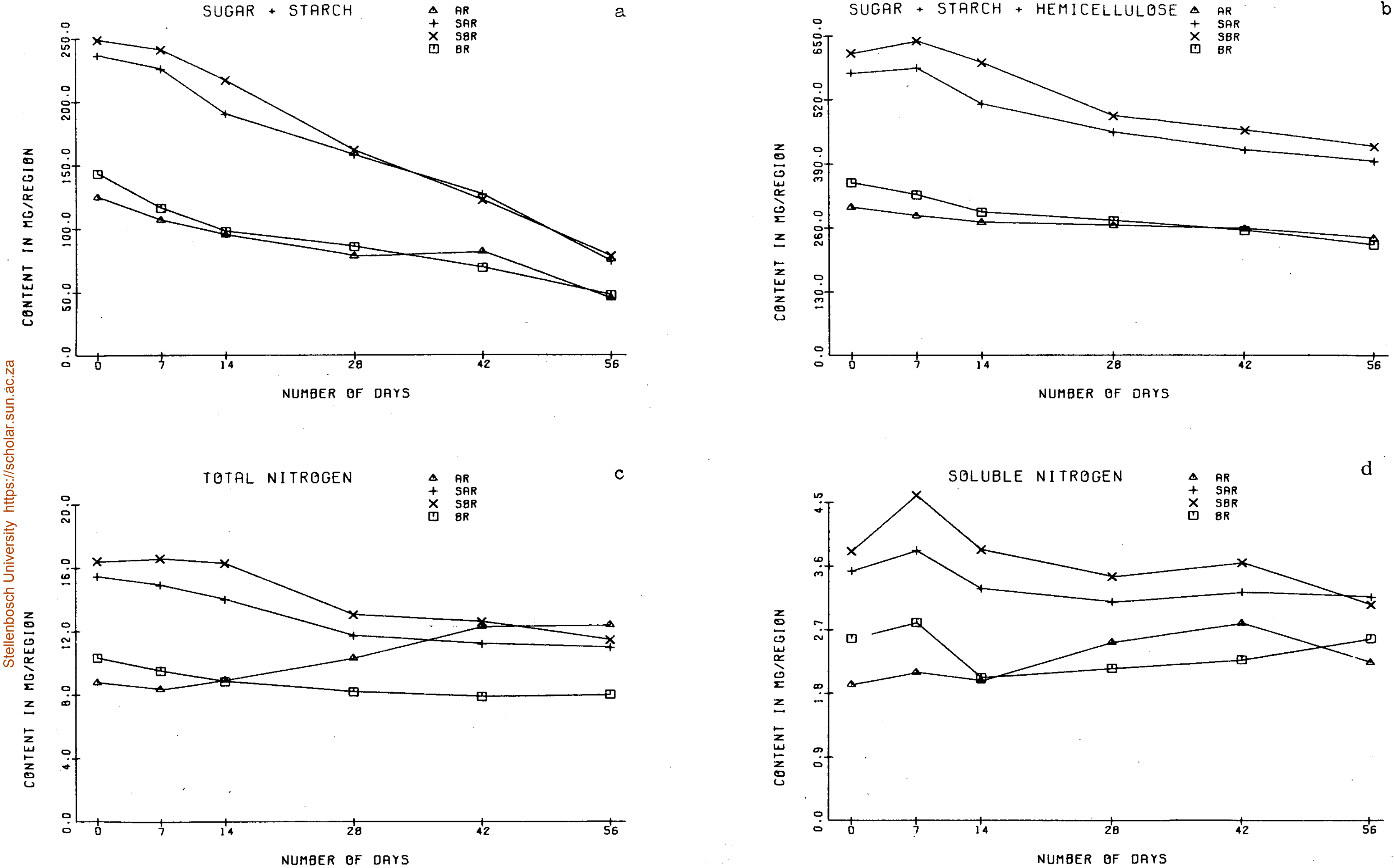


FIG. 48 - Effect of growth period on sugar + starch (a), sugar + starch + hemicellulose (b), total N (c), and soluble N (d) content in four regions of Jacquez plants treated with IBA

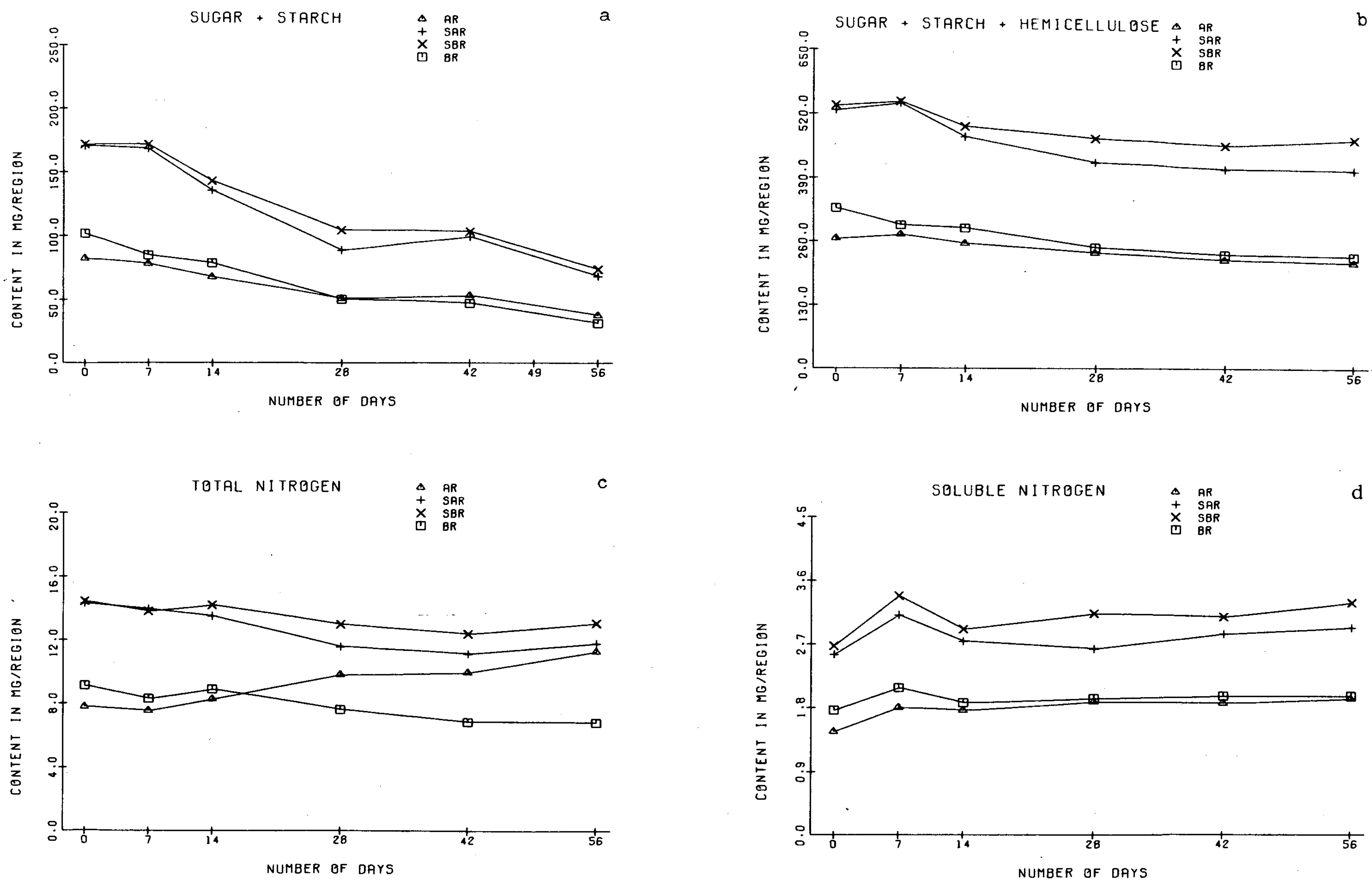


FIG. 49 - Effect of growth period on sugar + starch (a), sugar + starch + hemicellulose (b), total N (c), and soluble N (d) content in four regions of Salt Creek control plants

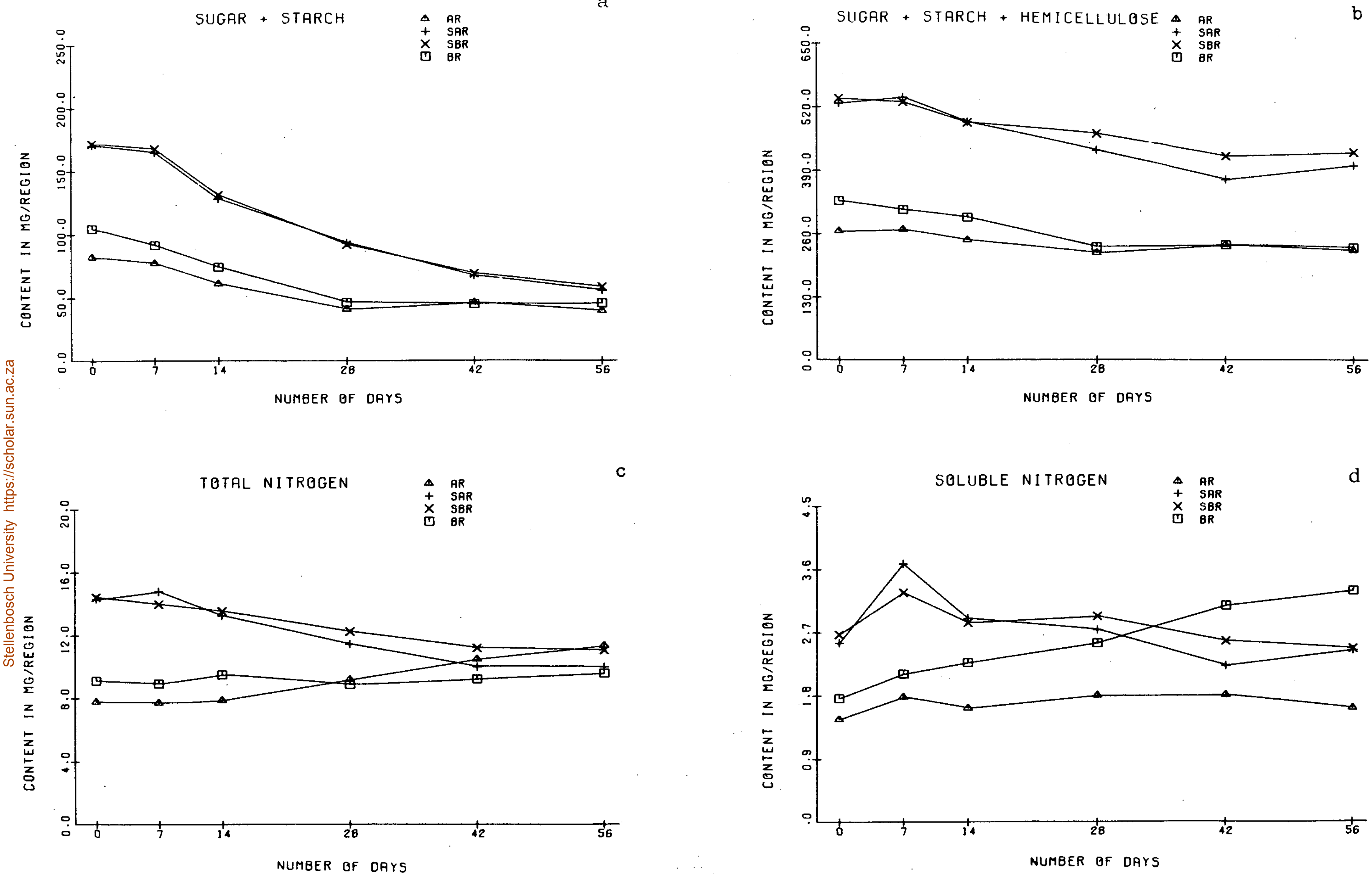


FIG. 50 - Effect of growth period on sugar + starch (a), sugar + starch + hemicellulose (b), total N (c), and soluble N (d) content in four regions of Salt Creek plants treated with IBA

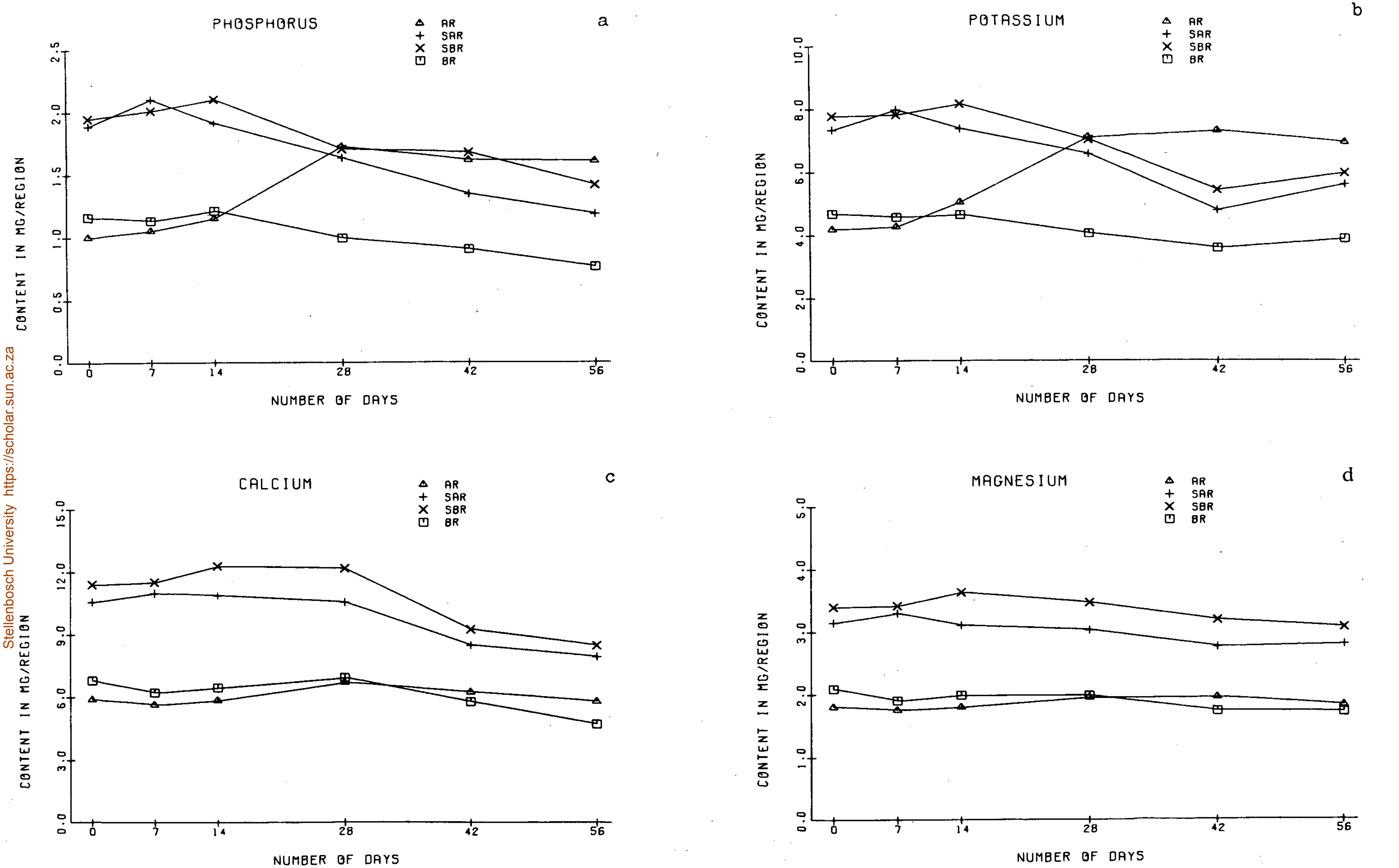


FIG. 51 - Effect of growth period on phosphorus (a), potassium (b), calcium (c), and magnesium (d) content in four regions of Jacquez control plants

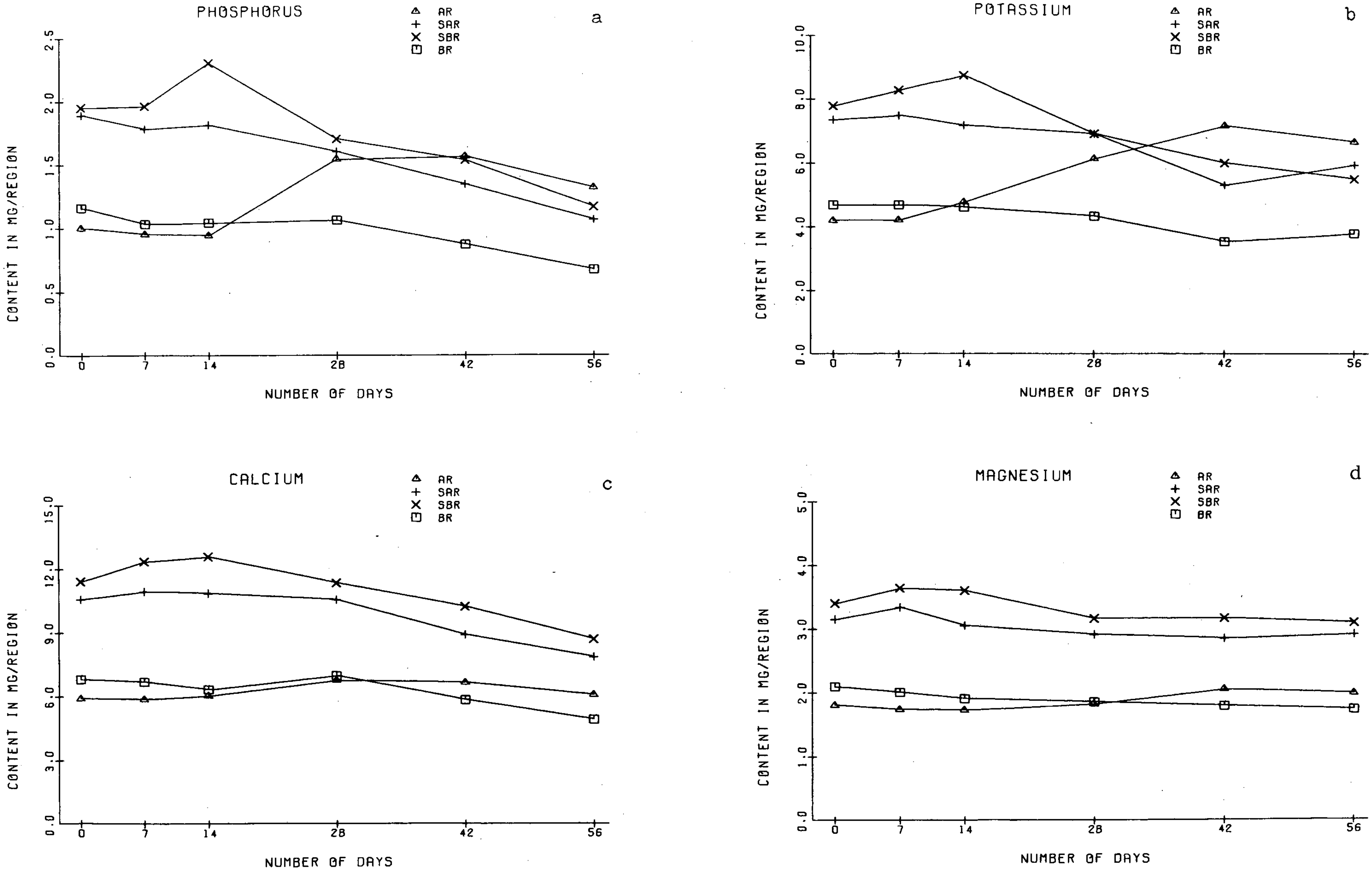


FIG. 52 - Effect of growth period on phosphorus (a), potassium (b), calcium (c), and magnesium (d) content in four regions of Jacquez plants treated with IBA

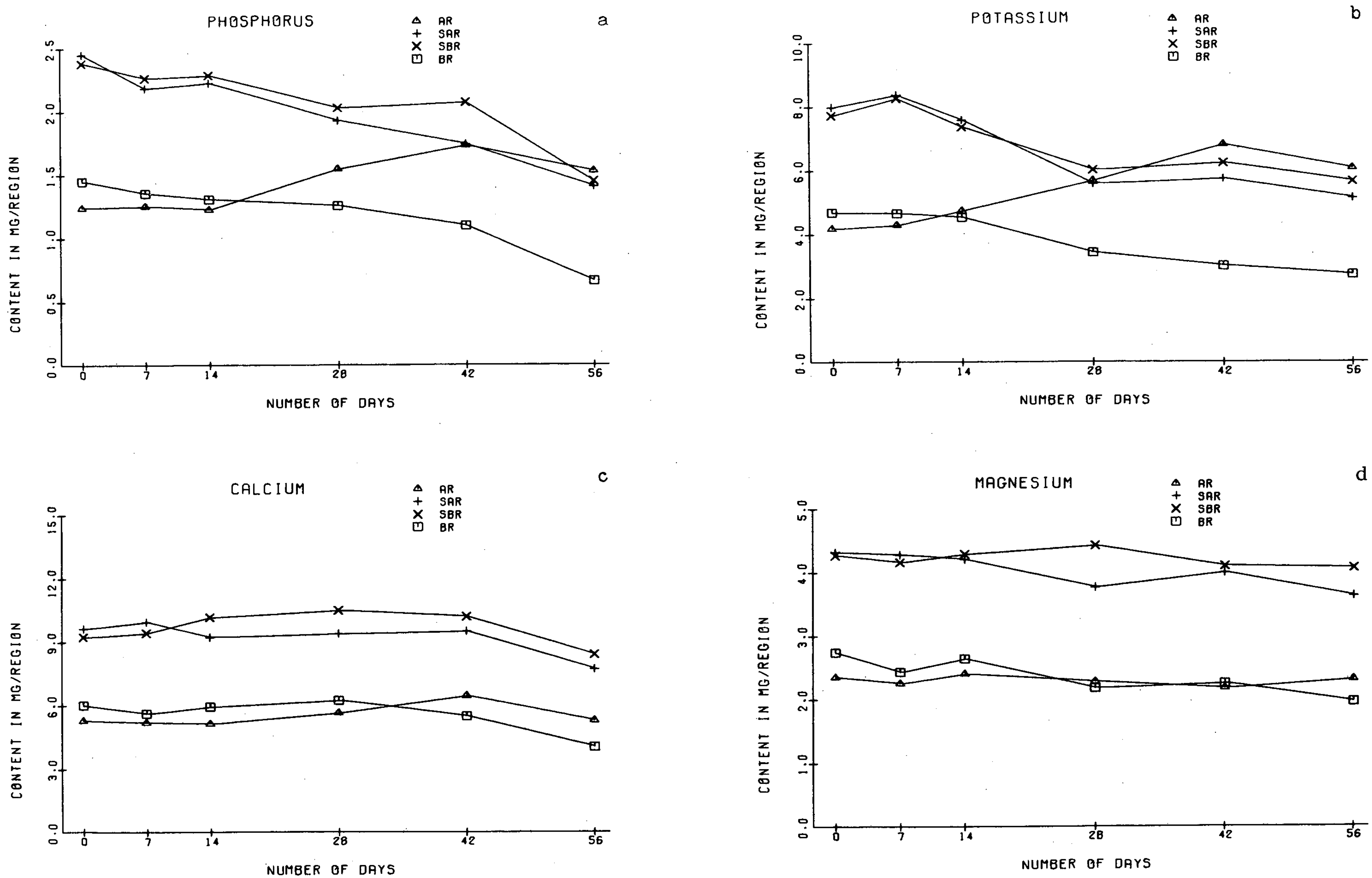


FIG. 53 - Effect of growth period on phosphorus (a), potassium (b), calcium (c), and magnesium (d) content in four regions of Salt Creek control plants

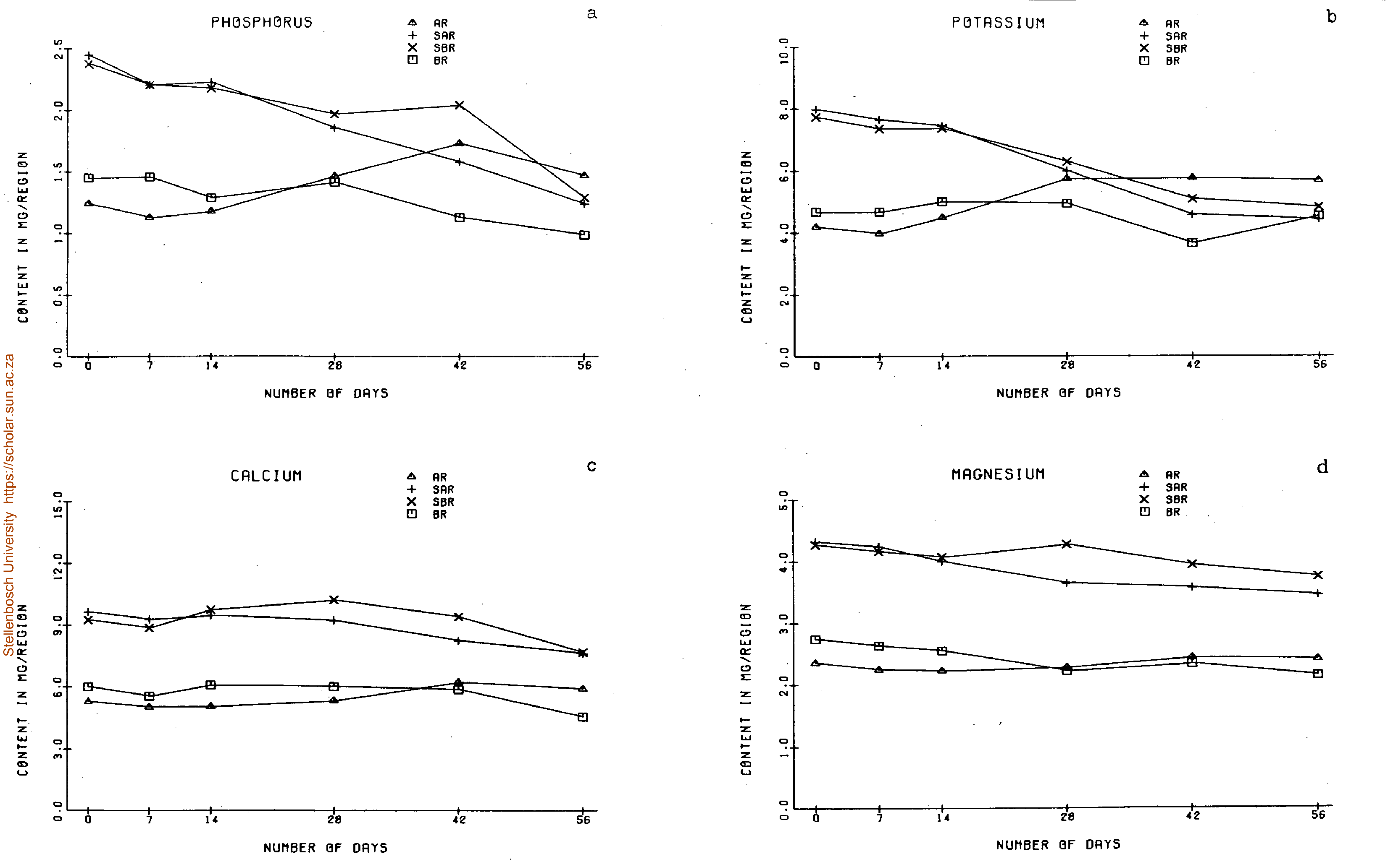


FIG. 54 - Effect of growth period on phosphorus (a), potassium (b), calcium (c), and magnesium (d) content in four regions of Salt Creek plants treated with IBA

TABLE 9 - Effect of growth period and IBA treatment on sugar content per region for Jacquez and Salt Creek plants

Rootstock and treatment	Region	Content (mg) Initially	Growing period (days) 7	14	28	42	56	Percentage distribution Initially	Finally
Jacquez Control	AR	46,42 C	a 43,58 C	a 29,10 AB	ab 23,78 AB	a 35,32 BC	a 17,25 A	15,6	12,5
	SAR	94,40 B	b 84,64 B	b 53,39 A	d 41,80 A	bc 55,75 A	bc 44,37 A	31,8	32,2
	SBR	102,50 D	b 89,34 C	bc 62,31 AB	e 49,65 A	c 64,06 B	cd 51,62 AB	34,5	37,4
	BR	53,79 C	a 45,25 BC	a 33,83 AB	bc 31,28 AB	a 37,17 AB	a 24,76 A	18,1	17,9
Jacquez IBA	AR	(46,42) C	a 36,78 B	a 29,16 AB	a 24,71 A	a 34,15 B	a 20,53 A	15,6	13,2
	SAR	(94,40) D	b 82,13 C	bc 55,59 D	cd 42,02 A	b 52,28 B	bc 42,53 A	31,8	27,4
	SBR	(102,50) E	b 86,12 D	c 64,42 C	cd 48,37 A	bc 57,96 BC	d 54,53 AB	34,5	35,1
	BR	(53,79) C	a 42,51 B	a 31,87 A	ab 31,35 A	a 36,38 AB	b 37,64 AB	18,1	24,3
Salt Creek Control	AR	31,29 AB	a 33,37 B	a 24,25 AB	a 20,77 A	a 25,79 AB	a 25,48 AB	15,8	17,2
	SAR	64,65 B	c 61,79 B	b 47,55 A	c 38,78 A	b 44,36 A	cd 46,65 A	32,6	31,5
	SBR	64,96 C	c 69,16 C	b 50,06 B	c 39,69 A	b 48,45 B	d 52,20 B	32,8	35,2
	BR	37,20 B	a 33,69 AB	a 27,82 AB	ab 23,20 A	a 27,46 AB	a 23,76 A	18,8	16,1
Salt Creek IBA	AR	(31,29) C	a 26,56 BC	a 23,71 AB	d 18,45 A	a 24,32 AB	a 23,10 AB	15,8	17,3
	SAR	(64,45) E	b 52,09 D	b 46,54 CD	bc 31,65 A	b 41,32 BC	bc 38,22 B	32,6	28,6
	SBR	(64,96) D	b 47,47 C	b 45,41 BC	c 39,02 AB	b 41,95 ABC	bc 37,40 A	32,8	28,0
	BR	(37,20) C	a 27,75 AB	a 29,14 AB	ab 25,97 A	a 30,78 ABC	b 34,82 BC	18,8	26,1

A - E : Compare in the same horizontal row

a - e : Compare in the same vertical column seperately for each cultivar

Means accompanied by a common letter are not different at the 5% level

TABLE 10 - Effect of growth period and IBA treatment on starch content per region for Jacquez and Salt Creek plants

Rootstock and treatment	Region	Content (mg)						Percentage distribution	
		Initially	Growing period (days)						
			7	14	28	42	56	Initially	Finally
Jacquez Control	AR	78,26 C	a 66,20 BC	a 65,40 BC	a 54,14 BC	ab 44,50 AB	ab 22,73 A	17,2	17,2
	SAR	141,7 D	b 137,70 D	b 136,60 D	b 97,00 C	c 71,88 B	bc 36,73 A	31,1	27,7
	SBR	146,0 B	b 143,20 B	b 142,50 B	c 119,40 B	c 69,68 A	c 52,14 A	32,1	39,4
	BR	89,35 B	a 75,10 B	a 72,34 B	a 45,00 A	a 27,54 A	ab 20,90 A	19,6	15,7
Jacquez IBA	AR	(78,26) D	a 69,60 CD	a 65,40 CD	a 53,16 BC	ab 46,73 B	ab 24,01 A	17,2	27,4
	SAR	(141,7) D	b 143,30 D	b 134,00 D	bc 115,10 C	c 73,30 B	ab 31,19 A	31,1	35,6
	SBR	(146,0) D	b 154,20 D	b 151,70 D	bc 112,50 C	bc 63,22 B	ab 22,96 A	32,1	26,2
	BR	(89,35)E	a 73,30 D	a 65,40 CD	a 53,80 C	a 31,96 B	a 9,50 A	19,6	10,8
Salt Creek Control	AR	50,70 C	a 44,20 C	a 43,00 C	a 29,70 B	a 27,11 B	a 12,26 A	15,5	19,4
	SAR	105,70 D	b 105,80 D	b 87,33 C	b 49,50 B	b 54,50 B	a 21,66 A	32,3	34,3
	SBR	106,50 D	b 101,80 CD	b 92,19 C	b 64,06 B	b 54,35 B	a 21,42 A	32,5	33,9
	BR	64,29 D	a 50,53 C	a 50,11 C	a 26,88 B	a 19,44 B	a 7,80 A	19,7	12,4
Salt Creek IBA	AR	(50,70)B	a 50,50 B	a 37,20 AB	a 22,38 A	a 21,71 A	a 16,42 A	15,5	25,0
	SAR	(105,70)D	b 112,40 D	b 81,38 C	b 61,28 C	a 25,64 A	a 17,53 A	32,3	26,7
	SBR	(106,50)D	b 119,80 D	b 85,50 C	b 52,70 B	a 26,76 A	a 20,86 A	32,5	31,8
	BR	(64,29)C	a 63,40 C	a 44,89 B	a 20,35 A	a 14,06 A	a 10,84 A	19,7	16,5

A - E : Compare in the same horizontal row

a - c : Compare in the same vertical column seperately for each cultivar

Means accompanied by a common letter are not different at the 5% level

TABLE 11 - Effect of growth period and IBA treatment on sugar + starch content per region for Jacquez and Salt Creek plants

Rootstock and treatment	Region	Content (mg) Initially	Growing period (days) 7	14	28	42	56	Percentage distribution Initially	Finally
Jacquez Control	AR	124,7 D	a 109,8 CD	a 94,50 BC	a 77,90 B	a 79,80 B	a 39,97 A	16,6	14,8
	SAR	236,1 D	b 222,3 D	b 190,0 C	b 138,8 B	b 127,6 B	b 81,10 A	31,4	30,0
	SBR	248,4 E	b 232,6 E	bc 204,8 D	c 169,1 C	b 133,7 B	c 103,8 A	33,0	38,4
	BR	143,2 D	a 120,3 C	a 106,2 C	a 76,30 B	a 64,70 AB	a 45,60 A	19,0	16,8
Jacquez IBA	AR	(124,7) D	a 106,4 C	a 94,60 BC	a 77,90 B	a 80,90 B	a 44,50 A	16,6	18,3
	SAR	(236,1) E	b 225,4 E	b 189,6 D	c 157,1 C	b 125,6 B	b 73,70 A	31,4	30,4
	SBR	(248,4) E	b 240,3 E	c 216,1 D	c 160,9 C	b 121,1 B	b 77,50 A	33,0	31,9
	BR	(143,2) E	a 115,8 D	a 97,3 C	a 85,10 C	a 68,30 B	a 47,10 A	19,0	19,4
Salt Creek Control	AR	81,99 D	a 77,56 CD	a 67,25 C	a 50,47 B	a 52,90 B	ab 37,73 A	15,6	17,9
	SAR	170,30 D	b 167,6 D	b 134,9 C	b 88,30 B	b 98,90 B	c 68,31 A	32,4	32,3
	SBR	171,50 D	b 170,9 D	b 142,2 C	b 103,7 B	b 102,8 B	c 73,63 A	32,6	34,9
	BR	101,50 D	a 84,20 C	a 77,93 C	a 50,10 B	a 46,90 B	a 31,56 A	19,4	14,9
Salt Creek IBA	AR	(81,99) C	a 77,0 BC	a 60,90 AB	a 40,80 A	a 46,00 A	ab 39,43 A	15,6	19,8
	SAR	(170,30) D	b 164,4 D	b 127,9 C	b 92,90 B	a 67,00 A	bc 55,74 A	32,4	28,0
	SBR	(171,50) D	b 167,3 D	b 130,9 C	b 91,70 B	a 68,70 A	bc 58,26 A	32,6	29,3
	BR	101,50) C	a 91,10 C	a 74,00 B	a 46,30 A	a 44,80 A	ab 45,65 A	19,4	22,9

A - E : Compare in the same horizontal row

a - c : Compare in the same vertical column seperately for each cultivar
Means accompanied by a common letter are not different at the 5% level

TABLE 12 - Effect of growth period and IBA treatment on hemicellulose content per region for Jacquez and Salt Creek plants

Rootstock and treatment	Region	Content (mg)						Percentage distribution	
		Initially	Growing period (days)						
			7	14	28	42	56	Initially	Finally
Jacquez Control	AR	176,9 A	a 166,1 A	ab 182,0 A	a 178,5 A	a 173,0 A	a 168,1 A	16,3	17,3
	SAR	337,7 C	c 339,1 C	c 323,7 C	b 302,2 B	b 276,0 A	c 301,9 B	31,0	31,1
	SBR	365,5 C	cd 350,9 BC	d 365,9 C	d 343,8 B	d 337,0 AB	d 326,8 A	33,6	33,7
	BR	208,1 C	a 183,8 AB	b 197,5 BC	a 189,6 AB	a 177,2 A	a 172,9 A	19,1	17,9
Jacquez IBA	AR	(176,9) A	a 177,9 A	a 176,4 A	a 186,2 A	a 177,3 A	b 196,1 A	16,3	18,7
	SAR	(337,7) B	d 358,4 C	c 321,1 B	b 297,0 A	c 292,8 A	d 324,3 B	31,0	30,9
	SBR	(365,5) CD	e 398,0 E	d 378,5 D	c 326,5 A	d 337,9 AB	e 350,5 BC	33,6	33,4
	BR	(208,1) B	a 210,9 B	ab 194,4 AB	a 188,5 AB	a 186,7 AB	a 179,4 A	19,1	17,0
Salt Creek Control	AR	183,10 AB	a 196,1 B	a 188,4 AB	a 185,5 AB	a 169,5 A	a 179,6 AB	16,2	16,1
	SAR	357,00 C	c 374,1 D	c 338,4 B	b 333,1 B	c 309,9 A	b 338,9 B	31,6	30,4
	SBR	365,40 AB	c 374,1 B	cd 351,5 A	c 366,2 AB	d 354,1 A	d 396,7 C	32,3	35,6
	BR	226,00 C	ab 209,1 BC	b 209,4 BC	a 197,0 AB	ab 186,4 A	a 198,4 AB	19,9	17,9
Salt Creek IBA	AR	(183,10) A	a 191,4 A	a 186,1 A	a 179,7 A	ab 186,1 A	a 187,4 A	16,2	17,2
	SAR	(357,00) C	c 374,9 D	d 360,8 C	b 339,4 B	c 304,8 A	b 346,8 BC	31,6	31,7
	SBR	(365,40) AB	c 361,9 AB	cd 356,7 AB	c 374,4 B	d 351,3 A	c 371,4 B	32,3	34,0
	BR	(226,00) B	b 217,8 B	b 219,7 B	a 186,9 A	b 192,6 A	a 187,0 A	19,9	17,1

A - E : Compare in the same horizontal row

a - e : Compare in the same vertical column seperately for each cultivar
Means accompanied by a common letter are not different at the 5% level

TABLE 13 - Effect of growth period and IBA treatment on sugar + starch + hemicellulose content per region for Jacquez and Salt Creek plants

Rootstock and treatment	Region	Content (mg)						Percentage distribution	
		Initially	Growing period (days)						
			7	14	28	42	56	Initially	Finally
Jacquez control	AR	301,5 C	a 275,9 BC	a 276,5 BC	a 256,5 B	a 252,8 B	a 208,1 A	16,4	16,8
	SAR	573,8 D	d 561,4 D	c 513,7 C	b 441,0 B	b 403,7 A	c 383,0 A	31,2	30,9
	SBR	614,0 E	d 583,5 D	d 570,8 D	d 512,9 C	c 470,8 B	d 430,2 A	33,4	34,7
	BR	351,3 E	b 304,1 D	b 303,7 D	a 265,8 C	a 241,9 B	ab 218,6 A	19,0	17,6
Jacquez IBA	AR	(301,5) C	ab 284,3 BC	a 271,0 B	a 264,1 AB	a 258,2 AB	b 240,6 A	16,4	18,6
	SAR	(573,8) D	d 583,8 D	c 510,7 C	b 454,0 B	b 418,4 A	c 398,0 A	31,2	30,8
	SBR	(614,0) D	e 638,3 E	e 594,6 D	c 487,4 C	c 459,1 B	d 428,0 A	33,4	33,1
	BR	(351,3) E	c 326,7 D	ab 291,6 C	a 273,7 BC	a 255,0 B	ab 226,5 A	19,0	17,5
Salt Creek control	AR	265,10 C	a 273,6 C	a 255,6 C	a 236,0 A	a 222,4 B	a 217,3 B	16,0	16,4
	SAR	527,40 D	c 541,7 D	c 473,3 C	b 421,4 A	c 408,8 B	b 407,2 B	31,8	30,7
	SBR	536,90 D	c 545,0 D	c 493,8 C	c 470,0 A	d 456,9 B	d 470,3 B	32,4	35,5
	BR	327,50 D	ab 293,3 C	b 287,4 C	a 247,1 A	a 233,3 B	a 230,2 B	19,8	17,4
Salt Creek IBA	AR	(265,10) B	a 268,4 B	a 247,0 AB	a 220,5 A	a 232,2 A	a 226,8 A	16,0	17,6
	SAR	(527,40) E	c 539,4 E	c 488,7 D	b 432,4 C	b 371,7 A	b 402,5 B	31,8	31,2
	SBR	(536,90) C	c 529,3 C	c 487,6 B	c 466,1 B	c 420,0 A	c 429,6 A	32,4	33,3
	BR	(327,50) C	b 309,0 BC	b 293,8 B	a 233,2 A	a 237,4 A	a 232,7 A	19,8	17,9

A - E : Compare in the same horizontal row

a - e : Compare in the same vertical column seperately for each cultivar
Means accompanied by a common letter are not different at the 5% level

TABLE 14 - Effect of growth period and IBA treatment on sugar and sugar + starch + hemicellulose as concentration of initial dry mass in four regions of Jacquez and Salt Creek plants

Rootstock and treatment	Region	Percentage of initial dry mass					
		Sugar		Sugar + starch + hemicellulose		Loss between 14 and 28 days	
		Growth period (days)		Growth period (days)		Sugar	Sugar + starch + hemicellulose
		14	28	14	28		
Jacquez control	AR	a 2,86	a 2,14	a 27,20	a 23,11	0,72	4,09
	SAR	a 2,96	a 2,38	a 28,50	b 25,13	0,58	3,37
	SBR	a 3,10	a 2,49	a 28,41	b 25,70	0,61	2,71
	BR	a 3,07	a 2,80	a 27,52	ab 23,80	0,27	3,72
Jacquez IBA	AR	a 2,96	a 2,22	a 27,47	ab 23,74	0,74	3,73
	SAR	a 3,11	a 2,39	a 28,61	b 25,77	0,72	2,84
	SBR	a 3,08	a 2,50	a 28,41	b 25,24	0,58	3,17
	BR	a 3,00	a 2,80	a 27,49	ab 24,42	0,20	3,07
Salt Creek control	AR	a 2,39	a 1,94	a 25,25	ab 22,07	0,45	3,18
	SAR	a 2,56	a 2,18	a 25,52	b 23,71	0,38	1,81
	SBR	a 2,60	a 2,00	a 25,68	b 23,69	0,60	1,99
	BR	a 2,49	a 2,17	a 25,68	b 23,14	0,32	2,54
Salt Creek IBA	AR	a 2,35	a 1,76	a 24,48	a 20,99	0,59	3,49
	SAR	a 2,50	a 1,75	a 26,24	b 23,86	0,75	2,38
	SBR	a 2,41	a 1,97	a 25,92	b 23,55	0,44	2,37
	BR	a 2,52	a 2,42	a 25,42	ab 21,72	0,10	3,70

a , b : Compare in the same vertical column seperately for each cultivar. Means accompanied by a common letter are not different at the 5% level

TABLE 15 - Effect of growth period and IBA treatment on total nitrogen content per region for Jacquez and Salt Creek plants

Rootstock and treatment	Region	Content (mg)						Percentage distribution	
		Initially	Growing period (days)						
			7	14	28	42	56	Initially	Finally
Jacquez control	AR	8,75 AB	a 8,23 A	a 9,47 B	c 11,82 C	bc 11,87 C	bc 11,33 C	17,2	26,8
	SAR	15,45 C	c 15,46 C	b 14,14 B	c 11,98 A	b 11,34 A	bc 11,49 A	30,3	27,2
	SBR	16,42 C	c 15,66 C	c 15,38 C	d 13,30 B	d 13,13 B	bc 12,03 A	32,2	28,4
	BR	10,31 C	ab 9,03 B	a 9,32 B	a 8,36 AB	a 7,78 A	a 7,47 A	20,3	17,6
Jacquez IBA	AR	(8,75) A	a 8,35 A	a 8,92 A	b 10,30 B	bcd 12,28 C	c 12,38 C	17,2	29,0
	SAR	(15,45) C	c 14,92 C	b 14,00 B	c 11,70 A	b 11,19 A	b 10,95 A	30,3	25,6
	SBR	(16,42) C	d 16,59 C	d 16,27 C	d 13,01 B	cd 12,57 B	bc 11,43 A	32,2	26,7
	BR	(10,31) C	a 9,51 B	a 8,82 AB	a 8,15 A	a 7,87 A	a 8,00 A	20,3	18,7
Salt Creek control	AR	7,76 A	a 7,52 A	ab 8,26 A	b 9,82 B	b 9,95 B	c 11,30 C	17,0	26,3
	SAR	14,29 B	c 13,93 B	d 13,50 B	c 11,57 A	c 11,12 A	c 11,80 A	31,4	27,4
	SBR	14,42 C	c 13,80 BC	d 14,19 C	d 12,97 AB	d 12,38 A	d 13,07 AB	31,7	30,4
	BR	9.09 C	ab 8,28 BC	bc 8,80 C	a 7,62 AB	a 6,83 A	a 6,83 A	19,9	15,9
Salt Creek IBA	AR	(7,76) A	a 7,72 A	a 7,78 A	b 9,16 B	c 10,45 C	c 11,31 C	17,0	27,0
	SAR	(14,29) D	c 14,79 D	d 13,24 C	c 11,41 B	b 9,98 A	b 9,96 A	31,4	23,8
	SBR	(14,42) C	c 13,98 C	d 13,52 C	cd 12,21 B	c 11,16 A	c 11,02 A	31,7	26,3
	BR	(9,09) A	b 8,94 A	c 9,49 A	b 8,86 A	b 9,20 A	b 9,56 A	19,9	22,9

A - D : Compare in the same horizontal row

a - d : Compare in the same vertical column seperately for each cultivar
Means accompanied by a common letter are not different at the 5% level

TABLE 16 - Effect of growth period and IBA treatment on soluble nitrogen content per region for Jacquez and Salt Creek plants

Rootstock and treatment	Region	Content (mg) Initially	Growing period (days) 7	14	28	42	56	Percentage distribution Initially	Finally
Jacquez control	AR	1,92 A	a 1,96 A	a 2,51 AB	b 3,02 B	b 2,96 B	a 2,53 AB	16,2	21,3
	SAR	3,53 A	c 3,30 A	bc 3,50 A	c 3,65 A	bc 3,26 A	b 3,40 A	29,9	28,6
	SBR	3,81 A	cd 3,50 A	bc 3,67 A	d 4,12 A	d 3,81 A	b 3,55 A	32,2	29,9
	BR	2,56 A	a 2,05 A	a 2,26 A	a 2,58 A	a 2,36 A	a 2,41 A	21,7	20,2
Jacquez IBA	AR	(1,92) A	a 2,09 A	a 1,97 A	a 2,52 BC	b 2,79 C	a 2,24 AB	16,2	20,3
	SAR	(3,53) AB	d 3,82 B	b 3,28 A	b 3,09 A	bc 3,23 A	b 3,17 A	29,9	28,7
	SBR	(3,81) B	e 4,60 C	c 3,83 B	bc 3,45 B	cd 3,65 B	b 3,06 A	32,2	27,7
	BR	(2,56) BC	b 2,80 C	a 2,02 A	a 2,15 AB	a 2,27 AB	a 2,58 BC	21,7	23,3
Salt Creek control	AR	1,46 A	a 1,80 A	a 1,76 A	a 1,88 A	a 1,87 A	a 1,93 A	17,3	19,1
	SAR	2,55 A	b 3,11 B	c 2,74 AB	bc 2,63 A	c 2,85 AB	bc 2,94 AB	30,2	29,0
	SBR	2,67 A	bc 3,38 C	c 2,91 AB	c 3,13 BC	e 3,09 BC	c 3,29 BC	31,6	32,5
	BR	1,76 A	a 2,08 A	ab 1,87 A	a 1,93 A	a 1,97 A	a 1,97 A	20,9	19,4
Salt Creek IBA	AR	(1,46) A	a 1,78 A	a 1,61 A	a 1,80 A	a 1,81 A	a 1,63 A	17,3	16,5
	SAR	(2,55) AB	c 3,68 C	c 2,90 B	bc 2,74 AB	ab 2,23 A	b 2,46 AB	30,2	24,9
	SBR	(2,67) AB	bc 3,27 B	c 2,84 AB	bc 2,93 AB	bc 2,58 A	b 2,48 A	31,6	25,1
	BR	(1,76) A	a 2,11 AB	b 2,27 AB	b 2,55 B	c 3,09 C	c 3,30 C	20,9	33,5

A - C : Compare in the same horizontal row

a - e : Compare in the same vertical column seperately for each cultivar

Means accompanied by a common letter are not different at the 5% level

TABLE 17 - Effect of growth period and IBA treatment on phosphorus content per region for Jacquez and Salt Creek plants

Rootstock and treatment	Region	Content (mg)						Percentage distribution	
		Initially	Growing period (days)						
			7	14	28	42	56	Initially	Finally
	AR	1,00 A	a 1,05 A	a 1,15 A	b 1,72 B	bc 1,61 B	d 1,60 B	16,7	32,3
Jacquez control	SAR	1,89 BC	c 2,10 C	bc 1,91 BC	b 1,63 B	b 1,34 A	bc 1,18 A	31,5	23,8
	SBR	1,95 BC	bc 2,01 C	cd 2,10 C	b 1,70 B	c 1,67 B	cd 1,41 A	32,5	28,5
	BR	1,16 B	a 1,13 B	a 1,21 B	a 0,99 AB	a 0,90 AB	a 0,76 A	19,3	15,4
	AR	(1,00) A	a 0,95 A	a 0,94 A	b 1,57 C	bc 1,56 C	bc 1,31 B	16,7	31,2
Jacquez IBA	SAR	(1,89) D	b 1,78 CD	b 1,81 CD	b 1,60 C	b 1,34 B	b 1,06 A	31,5	25,2
	SBR	(1,95) C	bc 1,96 C	d 2,30 D	b 1,70 B	bc 1,53 B	bc 1,16 A	32,5	27,6
	BR	(1,16) C	a 1,03 BC	a 1,04 BC	a 1,06 BC	a 0,87 B	a 0,67 A	19,3	16,0
	AR	1,24 A	ab 1,25 A	a 1,22 A	a 1,54 AB	b 1,72 B	c 1,52 AB	16,5	30,3
Salt Creek control	SAR	2,45 C	c 2,18 C	b 2,22 C	b 1,92 B	b 1,73 B	c 1,40 A	32,6	27,9
	SBR	2,38 C	c 2,26 BC	b 2,28 BC	b 2,02 B	c 2,06 B	c 1,44 A	31,6	28,7
	BR	1,45 C	ab 1,35 BC	a 1,30 BC	a 1,25 BC	a 1,09 B	a 0,66 A	19,3	13,1
	AR	(1,24) AB	a 1,12 A	a 1,17 AB	a 1,46 BC	b 1,72 C	c 1,46 BC	16,5	29,5
Salt Creek IBA	SAR	(2,45) D	c 2,20 D	b 2,20 D	b 1,85 C	b 1,57 B	c 1,23 A	32,6	24,8
	SBR	(2,38) C	c 2,20 BC	b 2,17 BC	b 1,96 B	c 2,03 B	c 1,28 A	31,6	25,9
	BR	(1,45) C	b 1,45 C	a 1,28 BC	a 1,41 C	a 1,12 AB	b 0,98 A	19,3	19,8

A - D : Compare in the same horizontal row

a - d : Compare in the same vertical column seperately for each cultivar
Means accompanied by a common letter are not different at the 5% level

TABLE 18 - Effect of growth period and IBA treatment on potassium content per region for Jacquez and Salt Creek plants

Rootstock and treatment	Region	Content (mg)						Percentage distribution	
		Initially	Growing period (days)						
			7	14	28	42	56	Initially	Finally
Jacquez control	AR	4,19 A	a 4,27 A	a 5,05 B	b 7,12 C	d 7,34 C	c 6,98 C	17,5	31,0
	SAR	7,35 CD	b 7,98 D	bc 7,39 CD	b 6,58 C	b 4,80 A	b 5,64 B	30,6	25,1
	SBR	7,78 BC	b 7,82 BC	cd 8,17 C	b 7,04 B	bc 5,45 A	bc 6,00 A	32,4	26,7
	BR	4,68 B	a 4,57 B	a 4,65 B	a 4,05 AB	a 3,59 A	a 3,89 AB	19,5	17,2
Jacquez IBA	AR	(4,19) A	a 4,20 A	a 4,76 A	b 6,18 B	d 7,19 B	c 6,69 B	17,5	30,5
	SAR	(7,35) B	b 7,49 B	b 7,17 B	b 6,93 B	bc 5,30 A	bc 5,96 A	30,6	27,2
	SBR	(7,78) BC	b 8,28 C	d 8,75 C	b 6,93 B	c 6,00 A	b 5,51 A	32,4	25,1
	BR	(4,68) A	a 4,68 A	a 4,60 A	a 4,32 A	a 3,52 A	a 3,79 A	19,5	17,2
Salt Creek control	AR	4,18 A	a 4,30 A	a 4,75 A	bc 5,71 B	e 6,84 C	d 6,10 B	17,0	31,0
	SAR	7,99 BC	b 8,38 C	b 7,61 B	bc 5,61 A	cd 5,76 A	bcd 5,16 A	32,5	26,1
	SBR	7,73 BC	b 8,27 C	b 7,38 B	bc 6,04 A	de 6,26 A	cd 5,69 A	31,4	28,9
	BR	4,69 C	a 4,67 C	a 4,54 C	a 3,44 B	a 3,02 AB	a 2,75 A	19,1	14,0
Salt Creek IBA	AR	(4,18) A	a 3,97 A	a 4,48 A	bc 5,75 B	cd 5,79 B	cd 5,72 B	17,0	29,2
	SAR	(7,99) C	b 7,65 C	b 7,45 C	bc 6,00 B	b 4,59 A	b 4,45 A	32,5	22,7
	SBR	(7,73) C	b 7,35 C	b 7,37 C	c 6,30 B	bc 5,10 A	bc 4,85 A	31,4	24,8
	BR	(4,69) AB	a 4,67 AB	a 5,00 B	b 4,94 B	a 3,67 A	b 4,56 AB	19,1	23,3

A - D : Compare in the same horizontal row

a - e : Compare in the same vertical column seperately for each cultivar
Means accompanied by a common letter are not different at the 5% level

TABLE 20 - Effect of growth period and IBA treatment on magnesium content per region for Jacquez and Salt Creek plants

Rootstock and treatment	Region	Content (mg)						Percentage distribution	
		Initially	Growing period (days)						
			7	14	28	42	56	Initially	Finally
Jacquez control	AR	1,80 AB	a 1,75 A	a 1,80 AB	a 1,95 B	ab 1,96 B	a 1,84 AB	17,2	19,4
	SAR	3,15 B	b 3,30 C	b 3,11 B	b 3,03 B	c 2,77 A	b 2,82 A	30,2	29,7
	SBR	3,40 B	b 3,42 B	c 3,64 C	c 3,47 B	d 3,20 A	b 3,09 A	32,6	32,6
	BR	2,09 C	a 1,90 B	a 1,99 BC	a 1,98 D	a 1,74 A	a 1,73 A	20,0	18,3
Jacquez IBA	AR	(1,80) A	a 1,73 A	a 1,72 A	a 1,82 A	b 2,05 B	a 1,99 B	17,2	20,4
	SAR	(3,15) C	b 3,34 D	b 3,05 BC	b 2,91 AB	c 2,85 A	b 2,92 AB	30,2	29,9
	SBR	(3,40) B	c 3,64 C	c 3,60 C	b 3,16 A	d 3,17 A	b 3,10 A	32,6	31,8
	BR	(2,09) C	a 2,00 BC	a 1,90 AB	a 1,85 AB	ab 1,79 A	a 1,74 A	20,0	17,9
Salt Creek control	AR	2,35 A	a 2,25 A	ab 2,40 A	a 2,28 A	a 2,18 A	b 2,32 A	17,2	19,3
	SAR	4,32 B	c 4,28 B	cd 4,21 B	b 3,77 A	c 4,01 AB	cd 3,64 A	31,6	30,3
	SBR	4,27 A	c 4,16 A	d 4,29 A	c 4,43 A	c 4,11 A	e 4,08 A	31,2	34,0
	BR	2,74 C	ab 2,43ABC	b 2,64 BC	a 2,18 AB	a 2,26 AB	a 1,97 A	20,0	16,4
Salt Creek IBA	AR	(2,35) A	a 2,24 A	a 2,22 A	a 2,28 A	a 2,44 A	b 2,41 A	17,2	20,5
	SAR	(4,32) C	c 4,24 C	c 4,00 BC	b 3,65 AB	b 3,58 AB	c 3,46 A	31,6	29,4
	SBR	(4,27) A	c 4,16 A	cd 4,07 A	c 4,28 A	c 3,95 A	d 3,76 A	31,2	31,9
	BR	(2,74) B	b 2,63 AB	b 2,56 AB	a 2,22 AB	a 2,34 AB	ab 2,15 A	20,0	18,2

A - D : Compare in the same horizontal row

a - e : Compare in the same vertical column seperately for each cultivar
Means accompanied by a common letter are not different at the 5% level

SECTION 12

EFFECT OF GROWTH AND IBA TREATMENT ON THE RESPIRATION RATE OF DIFFERENT CUTTING REGIONS

12.1 Introduction

It was shown in section 7 that, after 21 days of callusing, there was a considerable difference in respiration rate between the different cutting regions. Cuttings used for rooting differ from those used for grafting in having a bud near the apex which could possibly affect the respiration rate in that region. In section 11 it was shown that IBA treatment of cuttings stimulated root development. The question arises al to whether there is also a stimulation of respiration in the different cutting re= gions. This study was undertaken to investigate these aspects.

12.2 Procedure

Cuttings for rooting were collected at the beginning of August 1979 and held at $0^{o}C$ until 4 October when the experiment was commenced. The experiment was laid out as a randomized block design with six replicates of 20 cuttings each.

Treatments were as follows.

Jacquez control

Jacquez treated with 1 000 mg/1 IBA

Salt Creek control

Salt Creek treated with 1 000 mg/1 IBA

IBA treatments were applied by dipping the basal 1 cm of the cuttings in the solution for 5 minutes. The cuttings of all the treatments were planted as a randomised block design in sand (3% clay) in a dark room at a constant temperature of 18°C to correspond with the average temperature of 17,6°C in section 11. On 25 October 1979 the cuttings were removed from the medium and divided into four regions as shown in Fig. 36. Preparation of the regions, measurements of CO_2 evolution and calculation of the respiration rates were carried out according to the methods described in section 7, the only exception being that the measurements of CO_2 evolution were carried out at 18°C. All measurements were carried out with the cutting regions in the dark to prevent photosynthesis.

12.3 Results and discussion

The respiration rates of four regions of the Jacquez and Salt Creek control and IBA-treated cuttings is presented in Table 21.1. The dry mass of the shoot growth (Table 21.2) shows that some shoot development had already taken place after 21 days. However, there had not been any root development at that stage.

From a comparison of the respiration rates obtained in this study with those in section 7, it can be deduced that the presence of a bud at the apex of the cutting had a drastic enhancing effect on the respiration rate of the AR. In the present study in all treatments, the BR had a considerably lower respiration rate than the AR, while the SAR and SBR showed the lowest rate and did not differ from each other very substantially.

Similarly to the results in section 7, the Salt Creek control had a significantly lower respiration rate in the AR and a higher rate in the BR than Jacquez. These results indicate that, in Salt Creek, the respiratory activity is directed more strongly towards the base of the cuttings than in Jacquez.

IBA treatment of both cultivars significantly stimulated respiration in the BR only. The respiration rates increased by 17, 1% and 14, 5% respectively in Jacquez and Salt Creek.

By comparison of the shoot dry mass in the present study (Table 21.2) and those in section 11 (Appendix 34.3) it can be deduced that the cuttings in these studies had reached more or less the same stage of development after 21 days. Table 14 (section 11) shows that, in both cultivars, the sugar concentration did not differ statistically between regions after 14 or 28 days growth. A loss of sugar occurred in all regions over this period. The same table shows that the sugar + starch + hemicellulose concentration did not differ statistically between regions after 14 days, but after 28 days the AR and BR of both cultivars showed a tendency to= wards lower concentration with some of the differences significant.

The magnitude of differences between respiration rates in the different regions are much more than those of the corresponding sugar + starch + hemicellulose losses between 14 and 28 days. As respiration can be regarded as the main reason for carbohydrate loss, this indicates that translocation of carbohydrates in the form of sugars from the SAR and SBR to the AR and BR must have occurred.

With regard to sink/source relationships the following deductions can be made: The AR containing the bud and to a lesser extent the BR formed a sink to which sugars were translocated while the SAR and SBR were the source of these sugars.

TABLE 21.1 - Effect of hormone treatments on the respiration rate of four regions of Jacquez and Salt Creek cuttings after growing at 18°C for 21 days

Region	Respiration rate (ng $CO_2 . s^{-1} . g$ dry mass $^{-1}$)			
	Jacquez control	Jacquez IBA treated	Salt Creek control	Salt Creek IBA treated
AR	c 103,7 C	c 107,2 C	c 83,9 B	c 90,3 B
SAR	a 27,6 A	a 26,9 A	a 32,9 AB	a 36,8 B
SBR	a 25,1 A	a 26,6 A	a 33,4 B	a 36,8 B
BR	b 38,6 A	b 45,2 B	b 55,2 C	b 63,2 D

TABLE 21.2 - Effect of hormone treatments on the dry mass of bud growth per cutting at the apex of Jacquez and Salt Creek cuttings after growing at 18°C for 21 days

Dry mass of growth (mg)			
Jacquez control	Jacquez IBA treated	Salt Creek control	Salt Creek IBA treated
59 A	57 A	47 A	42 A

a - c : Compare in the same vertical column

A - D : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

SECTION 13

SUMMARY

Several facets with regard to the accumulation and utilisation of reserve nutrients related to propagation of the grapevine were studied.

13.1 Seasonal changes in carbohydrate and nitrogen fractions in the shoots (canes) of the mother vine and the effect of the composition of the canes on the callusing, shoot and root growth performance of cuttings taken from them:

The developing shoots on Jacquez and Salt Creek mother vines were sampled at monthly intervals and divided into six regions. The seasonal changes in dry mass, sugar, starch, hemicellulose, total N and soluble N were recorded. On 2 August (Southern Hemisphere) samples of cuttings for callusing and rooting were taken from each of the six regions. The callusing performance and shoot and root growth were correlated with the various components.

Sugar + starch + hemicellulose as concentration of fresh mass increased in all shoot regions, reaching peaks on 3 May in Salt Creek and 1 June in Jacquez. This was followed by small losses in Jacquez during June and considerable losses in Salt Creek during May and June. During July general small increases took place.

Of the three carbohydrate fractions studied, hemicellulose was the first to start accumulating, reaching peaks on 2 February (% of fresh mass). This was followed by losses after which a second series of peaks was reached on 3 May in Salt Creek and 1 June in Jacquez. This was followed by considerable losses.

On the basis of sugar and starch concentrations of fresh mass, the season can be divided into two distinct stages. The first stage started after bud-break and con= tinued until end May - early June. During this stage sugar remained at a fairly constant level around 1-2%, the excess sugar being converted to starch and other products. The second stage which covered the period early June to early August was characterised by the conversion of starch to sugar during which sugar in= creased substantially while starch decreased. Throughout the season starch was generally higher in the primary shoot regions than in the secondary shoots. At the final date on 2 August sugar + starch concentration was considerably higher in Jacquez than in Salt Creek.

Initial high total N concentrations (based on fresh mass) decreased to low values around February and March, subsequently showing a steady increase until 2 August. Soluble N followed the same basic pattern as total N in Jacquez. In Salt Creek soluble N also decreased until 2 February, followed by a sudden increase until 1 March and a loss of the same magnitude until 1 April. From then onwards the increase was slower than that of Jacquez resulting in Salt Creek reaching only about 50% of the corresponding values recorded in Jacquez on 2 August.

The Jacquez cuttings from different regions showed no significant difference in callusing or shoot and root growth, nor did these show any statistically signifi= cant correlation with any of the variables. The Salt Creek cuttings from different regions showed a statistically significant difference in callus score and the latter was also positively correlated with cutting mass.

13.2 Effect of cutting date and cold storage of Jacquez and Salt Creek cuttings on the carbohydrate levels and callusing performance:

Cuttings were collected at the beginning of the months May to September and analysed and callused immediately. Other cuttings were collected on the first three dates and cold-stored at $0^{O}C$ until 1 August when they were callused or analysed for sugar, starch and hemicellulose. Best callusing at the apex was obtained in cuttings col= lected on 2 May, and second best in those collected on 1 September.

Callusing performance was affected to a lesser extent by carbohydrate concentration than by other undertermined factors. Cold storage generally depressed callusing performance, and the longer the period of storage the greater was its effect.

13.3 Effect of callusing period and degree of callusing on the levels of carbohydrate and nitrogen fractions and other macro-elements in different regions of cuttings and scions, as well as the respiration rate of cuttings after callusing for 21 days:

Apart from the initial sampling, cuttings of Jacquez and Salt Creek and scions of Waltham Cross were callused for 7, 14 and 21 days. The callusing cuttings and scions sampled after 14 and 21 days were separated into "good" and "poor" callusing groups. All sampled cuttings were then divided into four regions. The samples were analysed for sugar, starch, hemicellulose, total and soluble N, P, K, Ca and Mg, the analytical results being expressed as concentration of initial dry mass and as total content per cutting. Further groups of cuttings of Jacquez and Salt Creek were callused for 21 days, divided as above, and the respiration rate of each region at 25 ^{o}C subsequently determined.

The largest carbohydrate losses from the whole cutting or scion comprised sugar in Jacquez and sugar and starch in Salt Creek and Waltham Cross. Losses of sugar + starch + hemicellulose from Salt Creek were considerably higher than from Jacquez over the full period. During this period little change occurred in the total N con= tent, but that of soluble N increased substantially. With the exception of Mg in Salt Creek which increased marginally, P, K, Ca and Mg was lost from all three cultivars.

The pattern of changes in sugar, starch and hemicellulose concentration in the four regions of Jacquez and Salt Creek was basically similar which indicates that they were depleted or accumulated to the same extent in all regions. This further in= dicates that the higher respiration rates obtained in the BR and AR were partly maintained by the utilisation of sugars translocated from other regions. These sugars were immediately utilised for respiration and no accumulation took place.

Total N in Jacquez and Salt Creek showed increases in the BR during callusing, while the concentration of soluble N and other macro-elements hardly differed be= tween regions. The "good" and "poor" callusing groups exhibited few differences in respect of all the above-mentioned components.

13.4 Effect of callusing temperature on the degree of callusing and carbohydrate utilisation:

Apart from the initial sampling, cuttings of Jacquez and Salt Creek were callused at 15°, 20° and 25°C for 28 days after which the callus was scored and the cuttings analysed for sugar, starch and hemicellulose. Results were expressed as concen= tration of initial dry mass.

Callusing was best at 25°C, slightly poorer at 20°C and none occurred at 15°C. In both cultivars callusing was poorer at the apex than at the base, with Jacquez showing a tendency towards better callusing at the apex and poorer callusing at the base than in the case of Salt Creek. The loss of sugar + starch + hemicellulose was positively related to the callusing temperature, Salt Creek showing a lower percentage loss at the two lower temperatures than Jacquez; at 25°C, however, the percentage loss was about the same in both cultivars.

13.5 Effect of cutting length on callusing and carbohydrate utilisation:

Apart from the initial sampling, cuttings of Jacquez and Salt Creek of the following lengths were callused for 28 days at 25°C: 20-25 cm with buds, 8 cm with one bud in the middle, and internodes of 8, 4, 2 and 1 cm. Samples were analysed for sugar, starch and hemicellulose.

Final sugar concentration differed little between cutting lengths, but final starch con= centration was positively related to cutting length. The presence or absence of a bud in the 8 cm cuttings had no statistically significant influence on the utilisation of sugar + starch, or on callusing at the base or apex. Cutting length influenced callusing at the base, the shorter cuttings callusing less, bud had no effect on callusing at the apex.

13.6 Seasonal changes of carbohydrate fractions during the nursery stage:

Cuttings of Jacquez and Salt Creek grown in a sandy soil were sampled for analysis over the full growing season. The sampling dates were as follows: 19 August (before planting), 2, 16, 30 September, 14, 28 October, 11, 25 November, 22 December, 22 January, 23 February, 22 March, 24 May and 23 July. The samples were analysed for sugar, starch and hemicellulose and results expressed on an absolute basis as well as concentration of dry mass.

In both cultivars shoot growth started before root growth, but the latter developed at a greater rate and on 23 July the dry mass ratio of roots to shoots was 3, 2 : 1 for Jacquez and 1, 7 : 1 for Salt Creek. The cuttings were dependent on their sugar, starch and hemicellulose reserves for a period of 12 weeks after planting. Starch concentration in the stem (cutting) subsequently increased rapidly while sugar concentration remained at a constant low level. Hemicellulose increased concomitantly with starch in the stem. The roots were the most important part for starch storage, while in the shoots hemicellulose was the dominating of the three carbohydrate components.

13.7 Effect of growth period and IBA treatment on the levels of carbohydrate and nitrogen fractions and other macro-elements in different regions of cuttings (plants) as well as the respiration rate of the different regions after growing at 18^{o}C for 21 days:

The treatments consisted of control Jacquez and Salt Creek cuttings and others of which the basal 1 cm had been dipped in 1 000 mg/l IBA for 5 minutes. Apart from an initial sampling of untreated cuttings, cutting of all treatments were planted in acid-washed sand, to be sampled after 7, 14, 28, 42 and 56 days. Planting was done on 28 October at which stage the weather conditions were warmer, and this allowed the changes to be followed in a shorter period of time. Deionised water was used for irregation. All sampled plants were divided into four regions and in these the dry mass, sugar, starch, hemicellulose, total and soluble N, P, K, Ca and Mg were determined. Analytical results were expressed as content per re= gion and for the whole plant. Further groups of cuttings treated similarly were grown at 18°C in sand for 21 days, divided as above, and respiration rate of each region at 18°C in the dark subsequently determined.

The plants lost dry mass until the final sampling, the losses varying between 7, 6 and 10, 6%. IBA treatment had no significant effect on dry mass losses. The largest losses in the whole plant were incurred by sugar and starch, but some hemicellulose was also utilised, showing that at least part of the hemicellulose had served as a reserve carbohydrate. IBA treatment had very little effect on the utilisation of the carbohydrate fractions in the whole plant over the full period. In the whole plant there was a loss of total N, P, K, Ca and Mg. Soluble N in Jacquez showed little change, but in Salt Creek it increased during the first 7 days. IBA treat= ment hardly affected the loss of total N, P, K, Ca and Mg.

In all plant regions the sugar + starch + hemicellulose content was depleted to about the same extent over the full period, but there were some mutual transformations between the fractions resulting in some changes in the distribution of the fractions in the plant. For instance, sugar content in the basal regions changed little in the untreated cuttings but actually increased over the full period in the IBA, treated cuttings.

From a comparison of respiration rates after 21 days and the loss of sugar + starch + hemicellulose it can be deduced that a translocation of carbohydrates in the form of sugars from the middle to the apical and basal regions of the plants must have occurred. IBA treatment stimulated respiration in the BR of Jacquez and Salt Creek.

Three of the elements, N, P and K showed more mobility in the plant with trans= location mainly towards the apical region. Ca and Mg showed less mobility, but also a translocation in the same direction. IBA treatment of Jacquez had little effect on translocation of the mobile elements when compared to the control. In Salt Creek IBA stimulated translocation of these elements to the basal region. The sink/source relationship was therefore changed by the IBA treatment.

IBA treatment had a larger stimulatory effect on the formation of soluble N in the Jacquez than in the Salt Creek. This effect was stronger nearer to the base where the IBA had been applied.

REFERENCES

ALEXANDER, D.McE., 1957. Seasonal fluctuations in the N content of the Sultana vine. Aust. J. agric Res. 8, 162 - 178.

ALLEY, C.J., 1962. Factors affecting the rooting of grape cuttings 11. Growth regulators. Am. J. Enol. Vitic. 12, 185 - 190.

ALLEY, C.J. & PETERSON, J.E., 1977. Grapevine propagation. IX. Effects of temperature, refrigeration, and indole butyric acid on callusing, bud push, and rooting of dormant cuttings. Am. J. Enol. Vitic. 28, 1 - 7.

ALLEY, C.J., 1979. Grapevine propagation. XI. Rooting of cuttings: Effects of indolebutyric acid (IBA) and refrigeration on rooting. Am. J. Enol. Vitic. 30, 28 - 32.

ALMELA PONS, G. & TIZZIO, R., 1965. The effects of indolebutyric acid and biotin on the rooting of rootstocks in relation to the graft union. Hort. Abstr. 38, Ab= stract 7276, 934.

ANON., 1971. The selection and issue of promising Waltham Cross clones. In= formation bulletin No. 33, FFTRI, Stellenbosch.

ANTAGNOZZI, E., CARTECHINI, A. & PREZIOSI, P., 1968. The effect of bottom heat and synthetic growth substances on the rooting of the rootstocks Kober 5 BB and 420 A. Hort. Abstr. 40, Abstract 5874, 712.

BABÓ, A. von. & MACH, E., 1909. Vermehrung des Weinstockes. In: Handbuch des Weinbaues und der Kellerwirtschaft, I. Berlin: Parey.

BENNETT, J.P., 1924. The distribution of carbohydrate foods in the apricot tree. Proc. Am. Soc. hort. Sci. 21, 372 - 384.

BERNSTEIN, Z. & KLEIN, S., 1957. Starch and sugar in canes of summer pruned Vitis vinifera plants. J. exp. Bot. 8, 87 - 95.

BEYERS, E., 1962. Diagnostic analysis for deciduous fruit. S. Afr. J. agric. Sci. 5, 315 - 329.

BOSMAN, P.W.J., 1976. Barlinka on Salt Creek. In: Information bulletin No. 168, FFTRI, Stellenbosch.

BOWEN, J.E. & NONAKA, T., 1967. Automatical analysis in sugar cane crop con= trol. Automn. anal. Chem. New York: Technicon Instrum. Corp.

BRUTSCH, M., 1971. Rooting and early growth of Carya illinoensis (Wang.) K. Koch stem cuttings. M.Sc. (Agric.) Thesis, Univ. Natal.

BUTTROSE, M.S., 1966. Use of carbohydrate reserves during growth from cuttings of grapevine. Aust. J. biol. Sci. 19, 247 - 256.

CERNOMOREC, M.V., 1969. Changes in the main components of the carbohydrate complex in vine shoots in relation to frost resistance. Hort. Abstr. 16, Abstract 5887, 714.

EIFERT, J., PÁNCZÉL, M. & EIFERT, A., 1961. Änderung des Stärke - und Zuckergehaltes der Rebe während der Ruheperiode. Vitis 2, 257 - 265.

ESAU, K., 1948. Phloem structure in the grapevine, and its seasonal changes. Hilgardia 18, 217 - 296.

GAINES, T.P., 1973. Automated determination of reducing sugars, total sugars and starch in plant tissue from one weighed sample. J. Ass. off. agric. Chem. 56, 1419 - 1424.

GALET, P., 1956. Les hybrides Producteurs Directs. In: Cepages et Vignobles de France, Tome 1. Montpellier: Paul Déhan.

GARDNER, V.R., BRADFORD, F.C. & HOOKER, J.R., 1952. Individual elements. In: The fundamentals of fruit production. New York, Toronto, London: McGraw=Hill, Inc.

GEORGESCU, M. & ILIE, M., 1970. Changes in carbohydrate content in the woody parts of the vine in autumn and spring before bud burst. Hort. Abstr. 42, Abstract 913, 129.

GOUSSARD, P.G., 1972. Beworteling van Salt Creek met hormoonbehandeling. Die Wynboer No. 484.

GOUSSARD, P.G. & VAN DER MERWE, G.G., 1973. Ondersoek na bewortelings=probleme van Salt Creek. Die Wynboer No. 506, 7 - 9.

GRASMANIS, V.O. & NICHOLAS, D.J.D., 1967. A nitrate reductase from apple roots. Phytochem. 6, 217 - 218.

HARDY, P.J. & POSSINGHAM, J. V., 1969. Studies on translocation of metabolities in the xylem of grapevine shoots. J. exp. Bot. 20, 325 - 335.

HARTMAN, H.T. & KESTER, D.E., 1975. Theoretical aspects of grafting and budding. In: Plant propagation, 2 nd ed. Englewood Cliffs, New Jersey: Prentice Hall, Inc.

HILL-COTTINGHAM, D.G. & LLOYD-JONES, P., 1975. Nitrogen - 15 in apple nutrition investigations. J. Sci. Fd. Agric. 26, 165 - 173.

HOSOI, T., MACHIDA, H. & KUROGOUCHI, H., 1971. Changes in the nutritional constituents in cuttings during propagation I. Distribution of dry matter and mineral elements in hardwood cuttings of grape vine. J. hort. Ass. Japan 40, 110 - 114.

HOSOI, T., MACHIDA, H. & YOSHIDA, R., 1972. Changes in the nutritional constituents in cuttings during propagation II. Changes in nitrogen and carbo=hydrate contents in hardwood cuttings of grapevines. J. hort. Ass. Japan 41, 127 - 132.

JULLIARD, B., 1964. The effect of indolyl-B-acetic acid on the rooting of cuttings of Vitis berlandieri. Hort. Abstr. 35, Abstract 5304, 562.

KAMARKAR, D.V., 1934. The seasonal cycle of nitrogenous and carbohydrate materials in fruit trees. 1. The seasonal cycles of total nitrogen and soluble nitrogen compounds in the wood, bark and leaves portions of terminal shoots of apple trees under two cultural systems-grass plus annual spring nitrate and arable without nitrogenous fertilizer. J. Pomol. 12, 177 - 221.

KIRKBY, E.A., 1974. Recycling of potassium in plants considered in relation to ion uptake and organic acid accumulation. In: Plant analysis and fertilizer problems. Proceedings of the 7 th international colloqium on plant analysis and fertilizer problems. Edited by J. Wehrmann.

KLIEWER, W.M., 1967. Annual cyclic changes in the concentration of free amino acids in grapevines. Am. J. Enol. Vitic. 18, 126 - 137.

KOLEDA, I., 1962. Changes in the carbohydrates of vine shoots during the winter dormant period. Hort. Abstr. 33, Abstract 4761, 493.

KOLEDA, I., 1964. Changes in the carbohydrate content of vine shoots and buds during dormancy. Hort. Abstr. 35, Abstract 7400, 793.

KONDO, I.N. & PUDRIKOVA, L.P., 1969. The results of biochemical studies on vine shoots in the autumn and winter months. Hort. Abstr. 41, Abstract 3575, 440.

MARAIS, J.P., 1965. The determination of available carbohydrates in grasses. M.Sc. (Agric.) Thesis, Univ. Natal.

MARAIS, J.P., 1969. The Weinman method for determination of total available carbohydrates in plant material containing starch. Agroplantae 1, 47 - 50.

McBRIDE, C.H., 1967. Determination of secondary and minor plant nutrients in fertilizer by Atomic Absorption Spectophotometry: Third collaborative study. J. Ass. off. agric. Chem. 50, 401 - 407.

MURNEEK, A.E., 1942. Quantitative distribution of nitrogen and carbohydrates in apple trees. Res. Bull. Mo. agric. Exp. Stn No. 348.

NASSAR, A.R. & KLIEWER, W.M., 1966. Free amino acids in various parts of Vitis vinifera at different stages of development. Proc. Am. Soc. hort. Sci. 89, 281 - 294.

OBBINK, J.G., ALEXANDER, D. McE. & POSSINGHAM, J.V., 1973. Use of nitrogen and potassium reserves during growth of grape vine cuttings. Vitis 12, 207 - 213.

OLAND, K., 1959. Nitrogenous reserves of apple trees. Physiologia Pl. 12, 594 - 648.

OLAND, K., 1963. Changes in the content of dry matter and major nutrient elements of apple foliage during senescence and abscission. Physiologia Pl. 16, 682 - 694.

ORFFER, C.J., 1956. Ontogenetic and experimental studies on the establishment of the graft union in the grapevine. Ph. D. Thesis, Univ. California.

PRIESTLY, C.A., 1960. Seasonal changes in the carbohydrate resources of some six-year old apple trees. Rep. E. Malling Res. Stn 1959, 70 - 77.

REUTHER, G., CHENG, C-Y. & SCHNEIDER, F., 1971. Beziehungen unterschied= licher Lagerungsbedingungen von Schnittholz zur Physiologie der Winterruhe bei der Gattung Vitis. Weinberg Keller 18, 173 - 191.

SCHAEFER, H., 1978. Der Proteinstoffwechsel der Jungreben in der Rebschule. Weinberg Keller 25, 331 - 352.

SCHENK, W., 1969. Stimulation der Veredlungspartner als Massnahme zur Erhöhung der Ausbeute an pflanzfähigen Pfropfreben. Weinberg Keller 16, 13 - 30.

SHIMOYA, C., GOMIDE, C.J. & FORTES, J.M., 1971. Anatomical studies of rooting and callus formation in vine, Vitis spp., grafted cuttings. Hort. Abstr. 41, Abstract 8613, 1034.

SKENE, K.G.M., 1971. Hormone effects on sugar release from vine canes. Ann. Bot. 35, 277 - 286.

SMITH, I.E., 1972. Further studies on the physiology of rooting and survival of Carya illinoensis (Wang.) K. Koch stem cuttings. M.Sc. (Agric.) Thesis, Univ. Natal.

STASSEN, P.J.C., 1973. Koolhidraatreserwes en mobilisasie in Malus domestica Borkh. M.Sc. (Agric.) Thesis, Univ. Stellenbosch.

STASSEN, P.J.C., 1980. A study on the carbohydrate and nitrogen metabolism in Prunus persica. Ph.D. (Agric.) Thesis, Univ. Stellenbosch.

STEPANOVA, A.F., 1969. Effect of growth stimulants and vitamins on root and callus formation in vine grafts. Hort. Abstr. 40, Abstract 5872, 711.

TIZIO, R., 1962. Effect of indolebutyric acid and biotin on the rooting of vine cuttings. Hort. Abstr. 33, Abstract 4753, 492.

TURNER, W.W., 1969. Enzymatic determination of starch in feeds, feed ingre= dients, and feces. J. Ass. off. agric. Chem. 52, 956 - 958.

WEAVER, R.J. & McCUNE, S.B., 1960. Effects of overcropping Alicante Bouschet grapevines in relation to carbohydrate nutrition and development of the vine. Proc. Am. Soc. hort. Sci. 75, 341 - 353.

WEAVER, R.J., 1972. Dormancy. In: Plant growth substances in agriculture. San Francisco: W.H. Freeman & Co.

WEAVER, R.J., 1976. Growth of the vine. In: Grape growing. New York: John Wiley & Sons, Inc.

WINKLER, A.J. & WILLIAMS, W.O., 1938. Carbohydrate metabolism of Vitis vinifera: hemicellulose. Pl. Physiol., Wash. 13, 381 - 390.

WINKLER, A.J. & WILLIAMS, W.O., 1945. Starch and sugars of Vitis vinifera Pl. Physiol., Wash. 20, 412 - 429.

WINKLER, A.J., Cook, J.A., KLIEWER, W.M. & LIDER, L.A., 1974. General Viticulture, 2nd ed. Berkley, Los Angeles & London: University of California Press.

APPENDIX 1.1 - Seasonal changes in the percentage dry mass in different Jacquez shoot regions

Region	Percentage dry mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3 5	1.6	1.7	2.8
BRP	10,7	17,4	b 27,2	bc 31,3	a 35,5	a 42,1	a 46,7	c 50,3	b 49,2	a 47,2	a 49,1
MRP	-	-	a 20,0	ab 27,5	a 35,0	a 41,1	a 47,1	bc 49,4	b 48,2	a 48,2	a 50,4
ARP	-	-	-	abc 29,6	a 33,8	a 37,0	a 45,7	bc 48,5	ab 45,8	a 49,5	a 51,8
BRS	-	-	a 18,9	a 26,8	a 33,5	a 35,6	a 42,4	a 44,1	ab 44,9	a 48,8	a 51,2
MRS	-	-	-	a 26,1	a 33,8	a 36,9	a 44,0	ab 45,1	ab 45,9	a 49,9	a 53,6
ARS	-	-	-	c 32,5	a 32,9	a 33,8	a 43,4	abc 47,7	a 42,0	a 50,4	a 53,8

APPENDIX 1.2 - Seasonal changes in sugar concentration of dry mass in different Jacquez shoot regions

Region	Percentage of dry mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	11,51	6,85	a 5,19	ab 5,66	a 4,92	a 4,88	a 3,55	a 3,67	b 4,82	c 8,80	cd 9,34
MRP	-	-	a 5,36	ab 5,87	b 5,63	ab 5,27	ab 3,78	ab 3,97	b 5,00	c 9,16	d 9,49
ARP	-	-	-	ab 5,88	c 6,37	c 6,54	b 4,42	ab 4,21	b 4,86	b 7,80	cd 9,26
BRS	-	-	b 6,28	a 5,31	ab 5,36	b 5,78	ab 4,17	ab 3,93	a 3,83	a 6,66	bc 8,80
MRS	-	-	-	b 6,17	b 5,51	ab 5,19	ab 4,17	b 4,36	a 3,90	a 6,95	a 8,06
ARS	-	-	-	ab 5,71	b 5,81	b 5,56	b 4,43	b 4,36	a 3,92	a 6,77	a 8,64

APPENDIX 1.3 - Seasonal changes in sugar concentration of fresh mass in different Jacquez shoot regions

Region	Percentage of fresh mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	1,23	1,19	b 1,41	ab 1,77	a 1,74	a 2,05	a 1,65	a 1,85	b 2,37	c 4,14	ab 4,58
MRP	-	-	a 1,07	ab 1,61	ab 1,97	ab 2,17	ab 1,78	a 1,96	b 2,41	d 4,41	b 4,78
ARP	-	-	-	ab 1,72	b 2,16	b 2,41	b 2,02	a 2,04	b 2,21	b 3,86	b 4,80
BRS	-	-	a 1,19	a 1,43	a 1,80	a 2,06	ab 1,76	a 1,73	a 1,72	a 3,25	ab 4,50
MRS	-	-	-	ab 1,61	ab 1,86	a 1,91	ab 1,83	a 1,96	a 1,80	a 3,46	a 4,32
ARS	-	-	-	b 1,86	ab 1,91	a 1,89	ab 1,92	a 2,07	a 1,66	a 3,42	ab 4,65

a - d : Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

APPENDIX 2.1 - Seasonal changes in percentage dry mass in different Salt Creek shoot regions

Region	Percentage dry mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	14,7	15,5	b 24,0	a 26,6	a 29,1	a 33,1	c 4,79	c 53,6	b 49,5	a 46,7	a 51,0
MRP	-	-	a 19,0	a 24,5	a 29,1	a 33,7	c 46,2	c 53,7	b 49,4	a 46,9	a 52,4
ARP	-	-	-	a 23,5	a 28,4	a 33,3	b 40,7	bc 50,9	b 49,2	a 47,7	ab 55,0
BRS	-	-	a 18,9	a 25,4	a 26,7	a 31,7	ab 37,4	ab 48,1	a 44,1	a 47,9	a 52,4
MRS	-	-	-	a 22,6	a 28,2	a 32,9	ab 36,8	a 46,6	a 42,5	a 48,9	b 57,2
ARS	-	-	-	a 23,7	a 26,5	a 33,8	a 33,5	a 46,4	a 43,9	a 49,6	b 58,2

APPENDIX 2.2 - Seasonal changes in sugar concentration of dry mass in different Salt Creek shoot regions

Region	Percentage of dry mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	7,30	4,90	a 4,39	b 5,06	ab 4,63	a 3,66	a 2,83	ab 3,62	a 3,67	b 6,38	ab 7,09
MRP	-	-	b 5,72	a 4,10	bc 4,93	a 3,69	ab 3,18	a 3,53	a 3,58	b 6,66	ab 7,28
ARP	-	-	-	b 5,19	ab 4,43	a 3,93	bc 3,68	abc 4,03	a 3,76	b 6,32	b 7,38
BRS	-	-	ab 5,04	a 4,30	a 4,27	a 3,67	bc 3,73	bc 4,18	a 3,50	ab 6,05	ab 7,12
MRS	-	-	-	a 4,47	bc 4,90	a 3,26	c 3,85	cd 4,44	a 3,22	a 5,68	ab 6,71
ARS	-	-	-	b 5,52	c 5,30	a 3,80	c 4,02	d 4,82	a 3,46	a 5,54	a 6,67

APPENDIX 2.3 - Seasonal changes in sugar concentration of fresh mass in different Salt Creek shoot regions

Region	Percentage of fresh mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	1,08	0,76	a 1,06	a 1,35	a 1,35	a 1,22	a 1,36	a 1,94	b 1,82	ab 2,98	a 3,62
MRP	-	-	a 1,08	a 1,00	a 1,44	a 1,24	a 1,47	a 1,90	b 1,77	b 3,12	ab 3,82
ARP	-	-	-	a 1,22	a 1,26	a 1,31	a 1,49	a 2,06	b 1,86	ab 3,02	b 4,06
BRS	-	-	a 0,95	a 1,09	a 1,14	a 1,17	a 1,39	a 2,01	ab 1,54	ab 2,89	ab 3,74
MRS	-	-	-	a 1,01	a 1,38	a 1,08	a 1,41	a 2,08	a 1,37	ab 2,78	ab 3,84
ARS	-	-	-	a 1,31	a 1,41	a 1,29	a 1,35	a 2,24	ab 1,53	a 2,75	ab 3,88

a - d : Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

APPENDIX 3.1 - Seasonal changes in starch concentration of dry mass in different Jacquez shoot regions

Region	Percentage of dry mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	3,91	2,76	a 1,86	a 2,77	a 3,36	b 7,23	c 10,96	d 10,66	c 10,66	b 7,69	b 5,59
MRP	-	-	a 2,21	a 2,64	a 3,52	b 6,63	c 10,37	cd 9,96	b 9,57	ab 7,13	b 5,64
ARP	-	-	-	a 3,18	b 4,62	a 5,17	b 8,95	bc 9,33	a 8,35	a 6,62	a 5,54
BRS	-	-	b 2,62	a 2,91	a 3,37	a 4,71	a 7,96	a 8,27	a 8,15	ab 6,83	b 5,36
MRS	-	-	-	a 3,57	a 3,43	a 5,35	a 7,77	ab 8,40	a 8,12	a 6,46	a 4,42
ARS	-	-	-	a 3,71	a 3,78	a 4,91	a 7,69	ab 9,00	a 7,66	a 6,24	a 4,57

APPENDIX 3.2 - Seasonal changes in starch concentration of fresh mass in different Jacquez shoot regions

Region	Percentage of fresh mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	0,42	0,48	a 0,50	a 0,87	a 1,20	b 3,07	c 5,12	d 5,37	d 5,25	a 3,63	a 2,75
MRP	-	-	a 0,44	a 0,73	a 1,23	b 2,76	c 4,89	cd 4,94	c 4,62	a 3,44	a 2,84
ARP	-	-	-	a 0,95	a 1,57	a 1,94	b 4,12	bc 4,52	b 3,88	a 3,28	a 2,88
BRS	-	-	a 0,50	a 0,78	a 1,14	a 1,70	a 3,38	a 3,68	ab 3,67	a 3,35	a 2,74
MRS	-	-	-	a 0,93	a 1,16	a 1,98	a 3,42	a 3,80	ab 3,74	a 3,22	a 2,37
ARS	-	-	-	a 1,21	a 1,24	a 1,74	a 3,36	b 4,30	a 3,22	a 3,16	a 2,46

APPENDIX 3.3 - Seasonal changes in sugar plus starch concentration of dry mass in different Jacquez shoot regions

Region	Percentage of dry masa at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	15,42	9,61	a 7,05	a 8,43	a 8,28	b 12,11	c 14,51	c 14,33	c 15,48	c 16,49	b 14,93
MRP	-	-	a 7,57	ab 8,51	ab 9,15	b 11,90	bc 14,15	bc 13,93	c 14,57	c 16,29	b 15,13
ARP	-	-	-	ab 9,06	c 10,99	b 11,71	b 13,37	bc 13,54	b 13,21	b 14,42	b 14,80
BRS	-	-	b 8,90	a 8,22	ab 8,73	a 10,49	a 12,13	a 12,20	a 11,98	ab 13,49	b 14,16
MRS	-	-	-	b 9,74	ab 8,94	a 10,54	a 11,94	ab 12,76	a 12,02	ab 13,41	a 12,48
ARS	-	-	-	ab 9,42	b 9,59	a 10,47	a 12,12	bc 13,36	a 11,58	a 13,01	a 13,21

APPENDIX 3.4 - Seasonal changes in sugar plus starch concentration of fresh mass in different Jacquez shoot regions

Region	Percentage of fresh mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3 5	1.6	1.7	2.8
BRP	1,65	1,67	b 1,91	a 2,64	a 2,94	c 5,12	b 6,77	d 7,22	c 7,62	b 7,77	ab 7,33
MRP	-	-	a 1,51	a 2,34	a 3,20	bc 4,93	b 6,67	cd 6,90	c 7,03	b 7,85	b 7,62
ARP	-	-	-	a 2,67	a 3,73	ab 4,35	b 6,14	cd 6,56	b 6,09	ab 7,14	b 7,68
BRS	-	-	a 1,69	a 2,21	a 2,94	a 3,76	a 5,14	a 5,41	ab 5,39	a 6,60	ab 7,24
MRS	-	-	-	a 2,54	a 3,02	a 3,89	a 5,25	ab 5,76	ab 5,54	a 6,68	a 6,69
ARS	-	-	-	a 3,07	a 3,15	a 3,63	a 5,28	bc 6,37	a 4,88	a 6,58	ab 7,11

a - d : Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

APPENDIX 4.1 - Seasonal changes in starch concentration of dry mass in different Salt Creek shoot regions

Region	Percentage of dry mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	4,64	2,93	a 2,06	a 1,93	ab 3,05	c 4,94	c 7,33	d 8,65	c 7,33	c 5,61	b 4,10
MRP	-	-	a 2,08	a 2,15	a 2,62	b 4,09	b 6,51	c 7,67	c 7,36	bc 5,20	ab 3,49
ARP	-	-	-	a 2,36	a 2,49	a 3,21	a 5,26	c 7,67	c 7,20	abc 4,99	ab 3,82
BRS	-	-	b 2,49	a 2,15	a 2,60	a 3,14	a 4,50	bc 7,24	b 6,16	abc 4,60	a 3,03
MRS	-	-	-	ab 2,74	b 3,84	a 2,99	a 4,25	ab 6,49	a 5,20	ab 4,29	a 2,90
ARS	-	-	-	b 3,38	b 3,65	a 2,96	a 4,54	a 6,22	ab 5,93	a 4,08	a 3,00

APPENDIX 4.2 - Seasonal changes in starch concentration of fresh mass in different Salt Creek shoot regions

Region	Percentage of fresh mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	0,68	0,45	b 0,50	a 0,51	a 0,89	a 1,63	d 3,51	e 4,63	b 3,64	a 2,62	a 2,09
MRP	-	-	a 0,39	a 0,53	a 0,77	a 1,38	c 3,02	d 4,12	b 3,63	a 2,43	a 1,82
ARP	-	-	-	a 0,56	a 0,71	a 1,07	b 2,17	cd 3,92	b 3,54	a 2,38	a 2,11
BRS	-	-	b 0,47	a 0,55	a 0,70	a 1,00	a 1,68	bc 3,49	a 2,72	a 2,20	a 1,60
MRS	-	-	-	a 0,62	a 1,08	a 0,99	a 1,58	ab 3,04	a 2,21	a 2,10	a 1,66
ARS	-	-	-	a 0,81	a 0,96	a 1,01	a 1,53	a 2,92	a 2,63	a 2,02	a 1,76

APPENDIX 4.3 - Seasonal changes in sugar plus starch concentration of dry mass in different Salt Creek shoot regions

Region	Percentage of dry mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	11,94	7,83	a 6,45	ab 6,99	a 7,68	c 8,60	c 10,16	a 12,27	c 11,00	c 11,99	b 11,19
MRP	-	-	b 7,80	a 6,25	a 7,55	bc 7,78	bc 9,69	a 11,20	c 10,94	c 11,86	ab 10,77
ARP	-	-	-	b 7,55	a 6,92	ab 7,14	ab 8,94	a 11,70	c 10,96	bc 11,31	b 11,20
BRS	-	-	b 7,53	ab 6,45	a 6,87	ab 6,81	a 8,23	a 11,42	b 9,66	ab 10,65	ab 10,15
MRS	-	-	-	ab 7,21	b 8,74	a 6,25	a 8,10	a 10,93	a 8,42	a 9,97	a 9,61
ARS	-	-	-	c 8,90	b 8,95	ab 6,76	a 8,56	a 11,04	b 9,39	a 9,62	a 9,67

APPENDIX 4.4 - Seasonal changea in sugar plus starch concentration of fresh mass in different Salt Creek shoot regions

Region	Percentage of fresh masa at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	1,76	1,21	a 1,56	a 1,86	a 2,24	a 2,85	b 4,87	c 6,57	b 5,46	a 5,60	a 5,71
MRP	-	-	a 1,47	a 1,53	a 2,21	a 2,62	b 4,49	bc 6,02	b 5,40	a 5,55	a 5,64
ARP	-	-	-	a 1,78	a 1,97	a 2,38	a 3,66	bc 5,98	b 5,40	a 5,40	a 6,17
BRS	-	-	a 1,42	a 1,64	a 1,84	a 2,17	a 3,07	ab 5,50	a 4,26	a 5,09	a 5,34
MRS	-	-	-	a 1,63	a 2,46	a 2,07	a 2,99	a 5,12	a 3,58	a 4,88	a 5,50
ARS	-	-	-	a 2,12	a 2,37	a 2,30	a 2,86	a 5,16	a 4,16	a 4,77	a 5,64

a - e : Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

APPENDIX 5.1 - Seasonal changes in hemicellulose concentration of dry mass in different Jacquez shoot regions

Region	Percentage of dry mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	8,22	20,84	b 24,04	c 26,61	cd 31,25	bc 26,65	ab 20,90	a 20,56	b 23,84	b 18,65	a 17,50
MRP	-	-	a 19,35	c 26,80	d 31,78	b 25,52	b 22,24	a 20,40	b 24,26	ab 17,67	a 16,99
ARP	-	-	-	b 22,82	b 29,27	b 26,29	a 19,91	a 19,99	b 24,76	a 16,51	a 16,25
BRS	-	-	a 18,09	b 23,27	b 29,18	c 28,02	a 19,47	a 19,50	a 21,82	a 16,66	a 16,27
MRS	-	-	-	a 21,17	bc 29,90	a 20,63	a 20,08	a 19,60	a 20,47	a 16,46	a 16,87
ARS	-	-	-	a 19,97	a 27,18	a 19,71	a 19,64	a 19,63	a 20,59	a 16,39	a 17,33

APPENDIX 5.2 - Seasonal changes in hemicellulose concentration of fresh mass in different Jacquez shoot regions

Region	Percentage of fresh mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	0,88	3,62	b 6,54	c 8,35	b 11,10	c 11,25	bc 9,75	b 10,34	c 11,72	a 8,79	a 8,60
MRP	-	-	a 3,87	b 7,37	b 11,11	bc 10,50	c 10,48	b 10,08	c 11,70	a 8,52	a 8,56
ARP	-	-	-	ab 6,71	ab 9,89	b 9,70	ab 9,11	ab 9,69	c 11,35	a 8,16	a 8,42
BRS	-	-	a 3,43	ab 6,25	a 9,77	b 9,98	a 8,25	a 8,61	b 9,81	a 8,14	a 8,33
MRS	-	-	-	a 5,52	ab 10,10	a 7,62	ab 8,85	a 8,84	ab 9,42	a 8,21	a 9,05
ARS	-	-	-	ab 6,49	a 8,92	a 6,70	a 8,52	ab 9,36	a 8,65	a 8,26	a 9,33

APPENDIX 5.3 - Seasonal changes in sugar plus starch plus hemicellulose concentration of dry mass in different Jacquez shoot regions

Region	Percentage of dry mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	23,64	30,44	b 31,09	c 35,03	bc 39,53	b 38,76	b 35,41	c 34,89	b 39,32	b 35,14	b 32,43
MRP	-	-	a 26,92	c 35,31	c 40,93	b 37,42	b 36,39	bc 34,33	b 38,83	b 33,96	b 32,12
ARP	-	-	-	b 31,88	c 40,26	b 38,00	a 33,28	abc 33,53	b 37,97	a 30,93	ab 31,05
BRS	-	-	a 26,99	b 31,49	ab 37,91	b 38,51	a 31,60	a 31,70	a 33,80	a 30,15	ab 30,43
MRS	-	-	-	ab 30,91	abc 38,84	a 31,17	a 32,02	ab 32,36	a 32,49	a 29,87	a 29,35
ARS	-	-	-	a 29,39	a 36,77	a 30,18	a 31,76	abc 32,99	a 32,17	a 29,40	ab 30,54

APPENDIX 5.4 - Seasonal changes in sugar plus starch plus hemicellulose concentration of fresh mass in different Jacquez shoot regions

Region	Percentage of fresh mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	2,53	5,29	b 8,45	b 10,99	ab 14,04	c 16,37	cd 16,52	c 17,56	c 19,34	a 16,56	a 15,93
MRP	-	-	a 5,38	ab 9,71	b 14,31	bc 15,43	d 17,15	c 16,98	bc 18,73	a 16,37	a 16,18
ARP	-	-	-	ab 9,38	ab 13,62	b 14,05	bc 15,25	bc 16,25	b 17,44	a 15,30	a 16,10
BRS	-	-	a 5,12	a 8,46	ab 12,71	b 13,74	a 13,39	a 14,02	a 15,20	a 14,74	a 15,57
MRS	-	-	-	a 8,06	ab 13,12	a 11,51	ab 14,10	ab 14,60	a 14,96	a 14,89	a 15,74
ARS	-	-	-	ab 9,56	a 12,06	a 10,33	ab 13,80	bc 15,73	a 13,53	a 14,84	a 16,44

a - d : Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

APPENDIX 6.1 - Seasonal changes in hemicellulose concentration of dry mass in different Salt Creek shoot regions

Region	Percentage of dry mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	8,90	18,25	a 20,56	b 19,50	b 26,76	b 21,30	b 20,62	a 21,15	ab 21,36	a 16,45	a 16,14
MRP	-	-	a 18,13	b 19,90	bc 27,63	ab 20,23	b 21,34	a 20,60	a 20,25	a 17,18	a 16,72
ARP	-	-	-	a 15,80	b 26,01	ab 20,72	a 19,25	ab 22,28	b 22,43	a 18,01	a 17,30
BRS	-	-	a 18,09	a 17,38	a 24,56	ab 19,80	a 18,59	b 23,49	b 22,42	a 17,52	a 16,36
MRS	-	-	-	a 16,22	c 30,43	a 19,23	a 18,39	b 23,03	b 22,50	a 16,64	a 16,87
ARS	-	-	-	a 17,38	c 28,67	a 19,38	a 17,55	ab 21,86	ab 21,61	a 17,00	a 16,27

APPENDIX 6.2 - Seasonal changes in hemicellulose concentration of fresh mass in different Salt Creek shoot regions

Region	Percentage of fresh mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	1,31	2,82	b 4,94	b 5,18	ab 7,80	a 7,05	c 9,88	a 11,33	ab 10,58	a 7,68	a 8,23
MRP	-	-	a 3,46	ab 4,87	b 8,04	a 6,81	c 9,87	a 11,06	ab 10,00	a 8,05	ab 8,77
ARP	-	-	-	a 3,71	ab 7,42	a 6,89	b 7,84	a 11,36	b 11,06	a 8,59	ab 9,52
BRS	-	-	a 3,41	ab 4,41	a 6,58	a 6,28	ab 6,94	a 11,30	ab 9,88	a 8,39	ab 8,58
MRS	-	-	-	a 3,64	b 8,59	a 6,33	ab 6,78	a 10,74	a 9,57	a 8,13	b 9,69
ARS	-	-	-	ab 4,11	ab 7,63	a 6,54	a 5,88	a 10,18	a 9,53	a 8,44	ab 9,48

APPENDIX 6.3 - Seasonal changes in sugar plus starch plus hemicellulose concentration of dry mass in different Salt Creek shoot regions

Region	Percentage of dry mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	20,84	26,08	a 27,01	b 26,49	bc 34,44	c 29,90	b 30,78	ab 33,42	ab 32,36	ab 28,44	ab 27,33
MRP	-	-	a 25,93	b 26,16	35,18	b 28,01	b 31,03	a 31,80	a 31,19	b 29,04	ab 27,49
ARP	-	-	-	a 23,35	ab 32,93	b 27,86	a 28,19	ab 33,97	b 33,39	b 29,32	b 28,50
BRS	-	-	a 25,62	a 23,83	a 31,43	ab 26,61	a 26,82	b 34,91	ab 32,08	ab 28,17	ab 26,51
MRS	-	-	-	a 23,43	d 39,17	a 25,48	a 26,49	ab 33,96	a 30,92	a 26,61	ab 26,48
ARS	-	-	-	b 26,28	d 37,62	ab 26,14	a 26,11	ab 32,90	a 31,00	a 26,62	a 25,94

APPENDIX 6.4 - Seasonal changes in sugar plus starch plus hemicellulose concentration of fresh mass in different Salt Creek shoot regions

Region	Percentage of fresh mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	3,07	4,03	b 6,50	a 7,04	ab 10,04	a 9,90	c 14,75	c 17,90	c 16,04	a 13,28	a 13,94
MRP	-	-	a 4,93	a 6,40	ab 10,25	a 9,43	c 14,36	abc 17,08	bc 15,40	a 13,60	a 14,41
ARP	-	-	-	a 5,49	ab 9,39	a 9,27	b 11,50	bc 17,34	c 16,46	a 13,99	a 15,69
BRS	-	-	a 4,83	a 6,05	a 8,42	a 8,45	a 10,01	abc 16,80	ab 14,14	a 13,48	a 13,92
MRS	-	-	-	a 5,27	b 11,05	a 8,40	a 9,77	ab 15,86	a 13,15	a 13,01	a 15,19
ARS	-	-	-	a 6,23	ab 10,00	a 8,84	a 8,74	a 15,34	a 13,69	a 13,21	a 15,12

a - c : Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

APPENDIX 7.1 - Seasonal changes in total nitrogen concentration of dry mass in different Jacquez shoot regions

Region	Percentage of dry mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	2,278	1,800	a 0,830	a 0,570	ab 0,490	a 0,402	a 0,370	a 0,472	a 0,625	a 0,688	a 0,711
MRP	-	-	c 1,465	a 0,628	a 0,468	ab 0,453	ab 0,402	a 0,495	a 0,684	a 0,733	a 0,757
ARP	-	-	-	bc 0,847	ab 0,550	b 0,524	ab 0,435	a 0,474	a 0,649	a 0,755	a 0,773
BRS	-	-	b 1,210	b 0,752	ab 0,506	ab 0,446	ab 0,384	a 0,465	a 0,631	a 0,764	a 0,797
MRS	-	-	-	bc 0,828	ab 0,505	ab 0,471	ab 0,418	a 0,464	a 0,681	a 0,747	a 0,754
ARS	-	-	-	c 0,902	b 0,596	b 0,534	b 0,486	a 0,502	a 0,705	a 0,791	a 0,787

APPENDIX 7.2 - Seasonal changes in total nitrogen concentration of fresh mass in different Jacquez shoot regions

Region	Percentage of fresh mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	0,243	0,313	a 0,224	a 0,178	a 0,173	a 0,170	a 0,172	a 0,238	a 0,307	a 0,325	a 0,350
MRP	-	-	b 0,292	a 0,172	a 0,163	a 0,187	a 0,190	a 0,245	a 0,330	ab 0,354	ab 0,381
ARP	-	-	-	b 0,251	a 0,186	a 0,194	a 0,198	a 0,230	a 0,301	ab 0,374	b 0,401
BRS	-	-	a 0,228	a 0,202	a 0,170	a 0,159	a 0,163	a 0,206	a 0,284	ab 0,374	b 0,408
MRS	-	-	-	ab 0,216	a 0,170	a 0,173	a 0,184	a 0,210	a 0,312	ab 0,374	b 0,404
ARS	-	-	-	c 0,293	a 0,196	a 0,176	a 0,210	a 0,239	a 0,326	b 0,398	b 0,425

APPENDIX 7.3 - Seasonal changes in soluble nitrogen concentration of dry mass in different Jacquez shoot regions

Region	Percentage of dry mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	0,690	0,657	a 0,185	ab 0,154	a 0,100	a 0,044	a 0,071	a 0,108	a 0,164	a 0,138	a 0,188
MRP	-	-	b 0,395	a 0,144	a 0,076	a 0,051	a 0,086	a 0,098	a 0,178	a 0,134	a 0,196
ARP	-	-	-	ab 0,156	a 0,099	a 0,066	a 0,075	a 0,092	a 0,149	ab 0,153	a 0,168
BRS	-	-	a 0,256	b 0,197	a 0,090	a 0,081	a 0,059	a 0,090	a 0,165	bc 0,192	a 0,180
MRS	-	-	-	ab 0,153	a 0,085	a 0,086	a 0,066	a 0,090	a 0,178	ab 0,168	a 0,159
ARS	-	-	-	a 0,109	a 0,095	a 0,089	a 0,074	a 0,091	a 0,173	c 0,217	a 0,165

APPENDIX 7.4 - Seasonal changes in soluble nitrogen concentration of fresh mass in different Jacquez shoot regions

Region	Percentage of fresh mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	0,073	0,114	a 0,050	a 0,048	a 0,036	a 0,019	a 0,033	a 0,054	a 0,081	a 0,066	a 0,093
MRP	-	-	b 0,079	a 0,039	a 0,027	a 0,021	a 0,041	a 0,049	a 0,086	a 0,065	a 0,099
ARP	-	-	-	a 0,046	a 0,033	a 0,024	a 0,034	a 0,044	a 0,070	ab 0,076	a 0,087
BRS	-	-	a 0,048	a 0,053	a 0,030	a 0,029	a 0,025	a 0,040	a 0,074	bc 0,094	a 0,092
MRS	-	-	-	a 0,040	a 0,029	a 0,032	a 0,029	a 0,041	a 0,082	ab 0,084	a 0,086
ARS	-	-	-	a 0,035	a 0,031	a 0,033	a 0,031	a 0,044	a 0,081	c 0,109	a 0,089

a - c : Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

APPENDIX 8.1 - Seasonal changes in total nitrogen concentration of dry mass in different Salt Creek shoot regions

Region	Percentage of dry mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	2,161	2,382	a 1,328	a 1,006	a 0,535	a 0,558	a 0,422	a 0,476	a 0,627	a 0,677	a 0,710
MRP	-	-	c 1,802	b 1,116	a 0,532	a 0,559	a 0,416	a 0,454	a 0,592	a 0,648	a 0,632
ARP	-	-	-	c 1,493	b 0,682	a 0,559	a 0,447	a 0,479	a 0,581	a 0,647	a 0,665
BRS	-	-	b 1,650	b 1,167	b 0,662	a 0,535	a 0,436	a 0,407	a 0,566	a 0,638	a 0,653
MRS	-	-	-	a 0,988	b 0,698	a 0,545	a 0,433	a 0,408	a 0,526	a 0,668	a 0,660
ARS	-	-	-	b 1,118	c 0,859	a 0,599	a 0,490	a 0,430	a 0,572	a 0,654	a 0,720

APPENDIX 8.2 - Seasonal changes in total nitrogen concentration of fresh mass in different Salt Creek shoot regions

Region	Percentage of fresh mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	0,318	0,368	a 0,318	b 0,268	a 0,156	a 0,183	a 0,202	b 0,255	c 0,311	a 0,316	a 0,361
MRP	-	-	a 0,339	b 0,274	a 0,155	a 0,188	a 0,192	b 0,244	bc 0,293	a 0,303	a 0,331
ARP	-	-	-	c 0,352	ab 0,194	a 0,186	a 0,182	b 0,243	bc 0,286	a 0,308	a 0,365
BRS	-	-	a 0,311	b 0,296	a 0,177	a 0,169	a 0,162	a 0,196	ab 0,251	a 0,305	a 0,342
MRS	-	-	-	a 0,224	ab 0,197	a 0,178	a 0,159	a 0,190	a 0,224	a 0,326	a 0,378
ARS	-	-	-	b 0,266	b 0,227	a 0,201	a 0,164	a 0,200	b 0,252	a 0,325	b 0,419

APPENDIX 8.3 - Seasonal changes in soluble nitrogen concentration of dry mass in different Salt Creek shoot regions

Region	Percentage of dry mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	,442	,748	b ,439	b 0,292	a 0,124	a 0,172	a 0,055	a 0,061	b 0,127	a 0,104	a 0,106
MRP	-	-	b ,474	b 0,296	a 0,123	a 0,158	a 0,058	a 0,061	ab 0,107	a 0,102	a 0,082
ARP	-	-	-	b 0,279	a 0,136	a 0,184	a 0,072	a 0,061	ab 0,097	a 0,107	a 0,108
BRS	-	-	a ,306	b 0,261	a 0,126	a 0,155	a 0,046	a 0,043	ab 0,101	a 0,091	a 0,083
MRS	-	-	-	a 0,199	a 0,127	a 0,172	a 0,055	a 0,046	a 0,069	a 0,076	a 0,088
ARS	-	-	-	a 0,182	a 0,138	a 0,181	a 0,065	a 0,046	a 0,066	a 0,072	a 0,090

APPENDIX 8.4 - Seasonal changes in soluble nitrogen concentration of fresh mass in different Salt Creek shoot regions

Region	Percentage of fresh mass at each date										
	1.10	3.11	1.12	2.1	2.2	1.3	1.4	3.5	1.6	1.7	2.8
BRP	0,065	0,115	b 0,105	b ,078	a ,036	a ,056	a ,026	a ,032	b ,063	a ,049	a ,054
MRP	-	-	b 0,090	b ,072	a ,036	a ,052	a ,026	a ,033	b ,053	a ,048	a ,043
ARP	-	-	-	b ,065	a ,038	a ,061	a ,029	a ,031	ab ,048	a ,051	a ,059
BRS	-	-	a 0,058	b ,066	a ,033	a ,048	a ,017	a ,021	ab ,045	a ,043	a ,043
MRS	-	-	-	a ,045	a ,036	a ,056	a ,020	a ,021	a ,029	a ,037	a ,050
ARS	-	-	-	a ,044	a ,037	a ,060	a ,022	a ,021	a ,029	a ,036	a ,053

a - c : Compare in the same vertical column. Means accompanied by a common letter are not different at the 5% level.

APPENDIX 9.1 - Effect of callusing period on sugar, starch, hemicellulose, total N and soluble N content in Jacquez and Salt Creek cuttings and Waltham Cross scions.

Cultivar	Component	Amount per cutting (mg)				Change from initial value after 21 days	
		Initially	Callusing period (days)			mg	%
			7	14	21		
Jacquez	Sugar	455,8 D	288,0 C	245,6 B	190,3 A	-265,5	-58,2
	Starch	256,3 A	285,5 B	316,2 C	282,5 B	+ 26,2	+10,2
	Sugar + starch	712,1 C	573,5 B	561,8 B	472,8 A	-239,3	-33,6
	Hemicellulose	765,9 A	868,3 B	880,3 B	850,8 B	+ 84,9	+11,1
	Sugar + starch + hemicellulose	1478,0 B	1441,8 B	1442,1 B	1323,6 A	-154,4	-10,4
	Total N	37,6 A	38,1 A	39,9 A	39,6 A	+ 2,0	+ 5,3
	Soluble N	8,9 A	10,9 B	11,0 B	11,6 B	+ 2,7	+30,3
Salt Creek	Sugar	266,4 C	158,7 B	116,1 A	114,3 A	-152,1	-57,1
	Starch	223,8 C	221,8 C	197,6 B	90,2 A	-133,6	-59,7
	Sugar + starch	490,2 D	380,5 C	313,7 B	204,6 A	-285,6	-58,3
	Hemicellulose	872,3 A	942,2 B	835,6 A	869,8 A	- 2,5	- 0,29
	Sugar + starch + hemicellulose	1362,5 C	1322,7 C	1149,3 B	1074,3 A	-288,2	-21,2
	Total N	36,8 A	37,3 A	34,6 A	35,1 A	- 1,7	- 4,6
	Soluble N	5,1 A	7,1 B	7,8 B	8,2 B	+ 3,1	+60,8
Waltham Cross	Sugar	120,0 C	41,9 A	42,5 A	57,3 B	- 62,7	-52,2
	Starch	67,6 B	96,6 D	84,1 C	45,6 A	- 22,0	-32,5
	Sugar + starch	187,6 D	138,5 C	126,6 B	102,9 A	- 84,7	-45,1
	Hemicellulose	207,7 A	248,0 D	234,6 C	220,7 B	+ 13,0	+ 6,3
	Sugar + starch + hemicellulose	395,3 D	386,5 C	361,2 B	323,6 A	- 71,7	-18,1
	Total N	8,4 B	8,5 B	8,5 B	8,0 A	- 0,4	- 4,8
	Soluble N	1,5 A	1,7 A	2,1 B	2,2 B	+ 0,7	+46,7

A - D : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 9.2 - Effect of callusing period on phosphorus, potassium, calcium and magnesium content in Jacquez and Salt Creek cuttings and Waltham Cross scions.

Cultivar	Component	Amount per cutting (mg)				Change from initial value after 21 days	
		Initially	Callusing period (days)			mg	%
			7	14	21		
Jacquez	Phosphorus	5,34 B	5,13 A	5,26 AB	5,17 A	-0,17	- 3,2
	Potassium	21,41 B	18,49 A	18,53 A	17,78 A	-3,63	-17,0
	Calcium	26,09 B	22,81 A	23,68 A	22,72 A	-3,37	-12,9
	Magnesium	8,53 A	8,54 A	8,46 A	8,33 A	-0,20	- 2,3
Salt Creek	Phosphorus	6,14 C	5,90 B	5,34 A	5,80 B	-0,34	- 5,5
	Potassium	19,87 A	18,62 A	18,15 A	18,24 A	-1,63	- 8,2
	Calcium	21,49 B	19,43 A	17,90 A	18,47 A	-3,02	-14,1
	Magnesium	11,06 B	10,45 A	10,18 A	11,18 B	+0,12	+ 1,1
Waltham Cross	Phosphorus	1,54 C	1,48 B	1,48 B	1,34 A	-0,20	-13,0
	Potassium	8,30 C	7,93 B	7,89 B	7,39 A	-0,91	-11,0
	Calcium	8,16 A	7,73 A	7,92 A	7,35 A	-0,81	- 9,9
	Magnesium	2,34 A	2,27 A	2,25 A	2,09 A	-0,25	-10,7

A - C : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 10.1 - Effect of callusing period on sugar concentration of initial dry mass in different regions of Jacquez cuttings

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 9,29 D	a 5,54 C	a 4,89 B	a 3,89 A
SAR	a 9,77 D	ab 6,04 C	bc 5,16 B	a 3,89 A
SBR	a 9,76 D	b 6,29 C	c 5,30 B	a 4,02 A
BR	a 9,11 D	ab 5,96 C	ab 5,01 B	a 4,02 A

a - c : Compare in the same vertical column

A - D : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 10.2 - Effect of callusing period and degree of callusing on sugar concentration of initial dry mass in different regions of Jacquez cuttings

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 4,63	a 4,79	a 4,86	a 4,56
	Poor	b 5,15	b 5,53	b 5,72	b 5,48
21	Good	a 3,88	a 3,84	a 3,79	a 3,88
	Poor	a 3,91	a 4,95	a 4,27	b 4,19

a - b : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 11.1 - Effect of callusing period on sugar concentration of initial dry mass in different regions of Salt Creek cuttings

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 5,11 C	a 3,15 B	a 2,32 A	a 2,35 A
SAR	b 5,63 C	a 3,33 B	a 2,40 A	a 2,44 A
SBR	b 5,56 C	a 3,24 B	a 2,38 A	a 2,26 A
BR	a 4,84 C	a 2,96 B	a 2,29 A	a 2,19 A

a - b : Compare in the same vertical column

A - C : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 11.2 - Effect of callusing period and degree of callusing on sugar concentration of initial dry mass in different regions of Salt Creek cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 2,41	a 2,43	a 2,44	a 2,31
	Poor	a 2,23	a 2,34	a 2,32	a 2,26
21	Good	a 2,39	a 2,52	a 2,28	b 2,42
	Poor	a 2,30	a 2,35	a 2,27	a 1,96

a - b : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 12.1 - Effect of callusing period on starch concentration of initial dry mass in different regions of Jacquez cuttings

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 5,06 A	a 5,08 A	a 5,96 B	a 4,79 A
SAR	a 5,10 A	b 6,22 B	a 6,87 B	b 6,15 B
SBR	a 5,28 A	b 6,11 B	a 6,38 B	b 6,12 B
BR	b 6,14AB	b 6,06AB	a 6,83 B	b 5,83 A

a - b : Compare in the same vertical column

A - B : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 12.2 - Effect of callusing period and degree of callusing on starch concentration of initial dry mass in different regions of Jacquez cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 5,88	a 6,82	b 6,72	a 6,71
	Poor	a 6,03	a 6,95	a 6,02	a 6,94
21	Good	a 4,70	a 5,93	a 6,23	a 5,91
	Poor	a 4,88	a 6,39	a 6,01	a 5,75

a - b : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 13.1 - Effect of callusing period on starch concentration of initial dry mass in different regions of Salt Creek cuttings

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 4,48 C	a 3,58 B	a 3,57 B	a 1,27 A
SAR	a 4,52 B	a 4,84 B	a 4,14 B	b 1,92 A
SBR	a 4,28 B	a 4,70 B	a 4,14 B	b 1,96 A
BR	a 4,97 C	a 4,15 B	a 3,81 B	b 1,83 A

a - b : Compare in the same vertical column

A - C : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 13.2 - Effect of callusing period and degree of callusing on sugar concentration of initial dry mass in different regions of Salt Creek cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 3,55	a 4,26	b 4,38	a 4,09
	Poor	a 3,62	a 4,03	a 3,91	a 3,57
21	Good	b 1,64	b 2,23	a 2,14	a 1,85
	Poor	a 0,80	a 1,56	a 1,76	a 1,78

a - b : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 14.1 - Effect of callusing period on sugar + starch concentration of initial dry mass in different regions of Jacquez cuttings

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 14,35 C	a 10,62 B	a 10,85 B	a 8,68 A
SAR	a 14,87 C	b 12,26 B	b 12,03 B	b 10,04 A
SBR	a 15,04 C	b 12,40 B	b 11,68 B	b 10,14 A
BR	a 15,25 C	b 12,02 B	b 11,84 B	b 9,85 A

a - b : Compare in the same vertical column

A - C : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 14.2 - Effect of callusing period and degree of callusing on sugar + concentration of initial dry mass in different regions of Jacquez cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 10,51	a 11,61	a 11,58	a 11,27
	Poor	a 11,18	b 12,48	a 11,74	a 12,42
21	Good	a 8,58	a 9,77	a 10,02	a 9,79
	Poor	a 8,79	a 10,34	a 10,28	a 9,94

a - b : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 15.1 - Effect of callusing period on sugar + starch concentration of initial dry mass in different regions of Salt Creek cuttings.

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 9,59 D	a 6,73 C	a 5,89 B	a 3,62 A
SAR	a 10,15 D	a 8,17 C	b 6,56 B	b 4,36 A
SBR	a 9,84 D	a 7,94 C	b 6,52 B	b 4,22 A
BR	a 9,81 D	a 7,11 C	ab 6,10 B	b 4,02 A

a - b : Compare in the same vertical column

A - D : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 15.2 - Effect of callusing period and degree of callusing on sugar + starch concentration of initial dry mass in different regions of Salt Creek cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 5,96	a 6,69	a 6,82	a 6,40
	Poor	a 5,85	a 6,37	a 6,23	a 5,83
21	Good	b 4,03	b 4,75	a 4,42	a 4,27
	Poor	a 3,10	a 3,91	a 4,03	a 3,74

a - b : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 16.1 - Effect of callusing period on hemicellulose concentration of initial dry mass in different regions of Jacquez cuttings.

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 16,84 A	a 18,07 A	a 17,91 A	a 17,47 A
SAR	a 16,31 A	a 18,20 B	a 18,58 B	a 17,68 B
SBR	a 15,78 A	a 18,11 B	a 18,02 B	a 18,01 B
BR	a 15,78 A	a 18,44 B	a 18,73 B	a 17,51 B

a : Compare in the same vertical column

A - B : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 16.2 - Effect of callusing period and degree of callusing on hemicellulose concentration of initial dry mass in different regions of Jacquez cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 17,64	a 18,34	a 17,96	a 18,57
	Poor	a 18,11	a 18,81	a 17,98	a 18,88
21	Good	a 17,68	a 17,70	a 18,17	a 17,46
	Poor	a 17,23	a 17,68	a 17,86	a 17,57

a : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 17.1 - Effect of callusing period on hemicellulose concentration of initial dry mass in different regions of Salt Creek cuttings.

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 17,95 AB	a 18,98 B	a 17,18 A	a 17,72 AB
SAR	a 17,66 A	a 19,09 B	a 17,00 A	a 17,83 A
SBR	a 17,09 A	a 19,02 B	a 16,89 A	a 17,69 A
BR	a 18,19 BC	a 18,85 C	a 16,37 A	a 17,24 AB

a : Compare in the same vertical column

A - C : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 17.2 - Effect of callusing period and degree of callusing on hemicellulose concentration of initial dry mass in different regions of Salt Creek cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 17,37	a 17,06	a 17,24	a 17,54
	Poor	a 17,02	a 17,82	a 16,55	a 16,88
21	Good	a 17,65	a 17,92	a 17,85	a 17,54
	Poor	a 17,82	a 17,73	a 17,50	a 16,88

a : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 18.1 - Effect of callusing period on sugar + starch + hemicellulose con-concentration of initial dry mass in different regions of Jacquez cuttings.

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 31,19 C	a 28,69 B	a 28,76 B	a 26,15 A
SAR	a 31,18 B	b 30,46 B	a 30,63 B	b 27,72 A
SBR	a 30,82 B	b 30,51 B	a 29,70 B	b 28,15 A
BR	a 31,03 B	b 30,42 B	a 30,57 B	b 27,36 A

a - b : Compare in the same vertical column

A - C : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level.

APPENDIX 18.2 - Effect of callusing period and degree of callusing on sugar + starch + hemicellulose concentration of initial dry mass in different regions of Jacquez cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 28,15	a 29,95	a 29,54	a 29,84
	Poor	a 29,29	b 31,29	a 29,72	b 31,30
21	Good	a 26,26	a 27,47	a 28,19	a 27,25
	Poor	a 26,02	a 28,02	a 28,14	a 27,51

a - b : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 19.1 - Effect of callusing period on sugar + starch + hemicellulose concentration of initial dry mass in different regions of Salt Creek cuttings.

Part	Concentration %			
	Initially	Callusing period (days)		
AR	a 27,54 D	a 25,71 C	a 23,07 B	a 21,34 A
SAR	a 27,81 C	b 27,26 C	a 23,54 B	a 22,19 A
SBR	a 26,93 C	ab 26,96 C	a 23,41 B	a 21,95 A
BR	a 28,00 D	ab 25,96 C	a 22,47 B	a 21,26 A

a - b : Compare in the same vertical column

A - D : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 19.2 - Effect of callusing period and degree of callusing on sugar + starch + hemicellulose concentration of initial dry mass in different regions of Salt Creek cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 23,33	a 23,75	a 24,06	b 23,46
	Poor	a 22,87	a 23,29	a 22,78	a 21,68
21	Good	a 21,68	a 22,67	a 22,27	b 21,81
	Poor	a 20,92	a 21,64	a 21,53	a 20,62

a - b : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 20.1 - Effect of callusing period on total N concentration of initial dry mass in different regions of Jacquez cuttings.

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 0,764 A	a 0,770 A	a 0,806 B	a 0,778 A
SAR	b 0,805 A	b 0,812 A	a 0,828 A	b 0,817 A
SBR	a 0,773 A	a 0,779 A	a 0,828 B	b 0,817 B
BR	b 0,806 A	b 0,827 A	b 0,861 B	c 0,878 B

a - c : Compare in the same vertical column

A - B : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level.

APPENDIX 20.2 - Effect of callusing period and degree of callusing on total N concentration of initial dry mass in different regions of Jacquez cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 0,815	b 0,853	a 0,841	b 0,889
	Poor	a 0,796	a 0,801	a 0,813	a 0,835
21	Good	a 0,790	a 0,821	b 0,833	a 0,893
	Poor	a 0,766	a 0,814	a 0,799	a 0,864

a - b : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 21.1 - Effect of callusing period on total N concentration of initial dry mass in different regions of Salt Creek cuttings.

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 0,714 B	a 0,746 C	a 0,675 A	a 0,665 A
SAR	b 0,745 B	a 0,750 B	b 0,698 A	a 0,692 A
SBR	ab 0,740 B	a 0,749 B	ab 0,682 A	a 0,688 A
BR	b 0,765 A	a 0,765 A	c 0,745 A	b 0,816 B

a - c : Compare in the same vertical column

A - C : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 21.2 - Effect of callusing period and degree of callusing on total N concentration of initial dry mass in different regions of Salt Creek cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 0,679	a 0,692	a 0,671	a 0,745
	Poor	a 0,671	a 0,705	a 0,691	a 0,746
21	Good	b 0,686	a 0,699	a 0,697	a 0,797
	Poor	a 0,641	a 0,685	a 0,681	a 0,833

a - b : Compare in the same vertical column seperately for each callusing period.

Means accompanied by a common letter are not different at the 5% level

APPENDIX 22.1 - Effect of callusing period on soluble N concentration of initial dry mass in different regions of Jacquez cuttings.

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 0,194 A	a 0,197 A	ab 0,229 B	a 0,232 B
SAR	a 0,204 A	a 0,221 AB	b 0,238 B	a 0,243 B
SBR	a 0,172 A	a 0,226 B	a 0,214 B	a 0,238 B
BR	a 0,183 A	b 0,265 B	b 0,243 B	a 0,249 B

a - b : Compare in the same vertical column

A - B : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 22.2 - Effect of callusing period and degree of callusing on soluble N concentration of initial dry mass in different regions of Jacquez cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 0,246	a 0,257	b 0,226	b 0,257
	Poor	a 0,212	a 0,221	a 0,202	a 0,229
21	Good	a 0,237	a 0,244	a 0,248	a 0,250
	Poor	a 0,228	a 0,242	a 0,226	a 0,249

a - b : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 23.1 - Effect of callusing period on soluble N concentration of initial dry mass in different regions of Salt Creek cuttings.

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 0,095 A	a 0,143 B	a 0,155 B	a 0,162 B
SAR	a 0,102 A	a 0,140 B	a 0,156 B	a 0,167 B
SBR	a 0,097 A	a 0,142 B	a 0,148 B	a 0,154 B
BR	a 0,117 A	a 0,154 B	b 0,172 BC	b 0,186 C

a - b : Compare in the same vertical column

A - C : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 23.2 - Effect of callusing period and degree of callusing on soluble N concentration of initial dry mass in different regions of Salt Creek cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 0,153	a 0,150	a 0,146	a 0,166
	Poor	a 0,157	a 0,163	a 0,151	b 0,178
21	Good	b 0,175	a 0,167	a 0,159	a 0,194
	Poor	a 0,163	a 0,167	a 0,150	a 0,178

a - b : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 24.1 - Effect of callusing period on phosphorus concentration of initial dry mass in different regions of Jacquez cuttings.

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 0,110 A	a 0,109 A	a 0,112 A	a 0,108 A
SAR	a 0,114 B	a 0,109 AB	a 0,109 AB	a 0,106 A
SBR	a 0,114 B	a 0,107 A	a 0,110 AB	a 0,106 A
BR	a 0,108 A	a 0,105 A	a 0,109 A	a 0,106 A

a : Compare in the same vertical column

A - B : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 24.2 - Effect of callusing period and the degree of callusing on phosphorus concentration of initial dry mass in different regions of Jacquez cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 0,114	a 0,111	a 0,107	a 0,108
	Poor	a 0,110	a 0,107	a 0,113	a 0,110
21	Good	a 0,111	a 0,105	a 0,107	a 0,104
	Poor	a 0,104	a 0,108	a 0,113	a 0,108

a : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 25.1 - Effect of callusing period on phosphorus concentration of initial dry mass in different regions of Salt Creek cuttings.

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 0,125 C	a 0,121 C	a 0,107 A	a 0,115 B
SAR	a 0,125 C	a 0,118 B	a 0,106 A	a 0,113 B
SBR	a 0,122 B	a 0,120 B	a 0,107 A	a 0,112 A
BR	a 0,123 B	a 0,117 A	a 0,113 A	b 0,134 C

a - b : Compare in the same vertical column

A - C : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 25.2 - Effect of callusing period and degree of callusing on phosphorus concentration of initial dry mass in different regions of Salt Creek cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 0,107	a 0,108	a 0,107	a 0,115
	Poor	a 0,107	a 0,105	a 0,107	a 0,110
21	Good	b 0,122	b 0,118	a 0,116	a 0,136
	Poor	a 0,109	a 0,108	a 0,109	a 0,133

a - b : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 26.1 - Effect of callusing period on potassium concentration of initial dry mass in different regions of Jacquez cuttings.

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 0,378 A	a 0,383 A	a 0,393 A	ab 0,384 A
SAR	b 0,420 C	a 0,394 BC	a 0,382 AB	a 0,359 A
SBR	b 0,417 B	a 0,383 A	a 0,379 A	a 0,357 A
BR	b 0,423 A	a 0,389 A	a 0,401 A	b 0,401 A

a - b : Compare in the same vertical column

A - C : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 26.2 - Effect of callusing period and degree of callusing on potassium concentration of initial dry mass in different regions of Jacquez cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 0,387	a 0,375	a 0,360	a 0,393
	Poor	a 0,400	a 0,390	b 0,398	a 0,410
21	Good	a 0,369	a 0,343	a 0,348	a 0,411
	Poor	a 0,401	a 0,376	a 0,367	a 0,392

a - b : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 27.1 - Effect of callusing period on potassium concentration of initial dry mass in different regions of Salt Creek cuttings.

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 0,377 A	a 0,378 A	a 0,366 A	a 0,359 A
SAR	a 0,410 B	a 0,371 A	a 0,349 A	a 0,359 A
SBR	a 0,410 A	a 0,379 A	b 0,394 A	a 0,383 A
BR	a 0,388 B	a 0,376 B	a 0,354 A	a 0,371 B

a - b : Compare in the same vertical column

A - B : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 27.2 - Effect of callusing period and degree of callusing on potassium concentration of initial dry mass in different regions of Salt Creek cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 0,377	a 0,359	a 0,394	a 0,354
	Poor	a 0,354	a 0,339	a 0,393	a 0,353
21	Good	a 0,357	a 0,360	a 0,377	a 0,362
	Poor	a 0,362	a 0,358	a 0,390	a 0,381

a : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 28.1 - Effect of callusing period on calcium concentration of initial dry mass in different regions of Jacquez cuttings.

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 0,472 A	a 0,478 A	a 0,487 A	a 0,507 A
SAR	b 0,552 B	a 0,477 A	a 0,498 A	a 0,450 A
SBR	b 0,560 B	a 0,479 A	a 0,483 A	a 0,475 A
BR	b 0,567 B	a 0,480 A	a 0,506 A	a 0,480 A

a - b : Compare in the same vertical column

A - B : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 28.2 - Effect of callusing period and degree of callusing on calcium concentration of initial dry mass in different regions of Jacquez cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 0,488	a 0,522	a 0,507	a 0,527
	Poor	a 0,485	a 0,475	a 0,462	a 0,485
21	Good	a 0,511	a 0,460	a 0,495	a 0,500
	Poor	a 0,503	a 0,439	a 0,457	a 0,461

a : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 29.1 - Effect of callusing period on calcium concentration of initial dry mass in different regions of Salt Creek cuttings.

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 0,395 B	a 0,358 AB	a 0,327 A	a 0,379 AB
SAR	a 0,442 B	ab 0,387 A	a 0,368 A	a 0,375 A
SBR	a 0,448 B	ab 0,387 A	a 0,361 A	a 0,370 A
BR	a 0,423 A	b 0,432 A	a 0,379 A	a 0,382 A

a - b : Compare in the same vertical column

A - B : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 29.2 - Effect of callusing period and degree of callusing on calcium concentration of initial dry mass in different regions of Salt Creek cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 0,320	a 0,356	a 0,362	a 0,379
	Poor	a 0,334	a 0,380	a 0,361	a 0,379
21	Good	a 0,375	a 0,369	a 0,365	a 0,366
	Poor	a 0,382	a 0,382	a 0,375	a 0,399

a : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 30.1 - Effect of callusing period on magnesium concentration of initial dry mass in different regions of Jacquez cuttings.

Part	Concentration %			
	Initially	Callusing period (days)		
		7	14	21
AR	a 0,172 A	a 0,165 A	a 0,175 A	a 0,167 A
SAR	a 0,180 A	a 0,187 A	a 0,178 A	a 0,172 A
SBR	a 0,180 A	a 0,178 A	a 0,175 A	a 0,178 A
BR	a 0,180 A	a 0,178 A	a 0,178 A	a 0,173 A

a : Compare in the same vertical column

A : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 30.2 - Effect of callusing period and degree of callusing on magnesium concentration of initial dry mass in different regions of Jacquez cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 0,180	a 0,177	a 0,173	a 0,177
	Poor	a 0,170	a 0,180	a 0,177	a 0,180
21	Good	a 0,171	a 0,171	a 0,181	b 0,181
	Poor	a 0,164	a 0,172	a 0,175	a 0,165

a - b : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 31.1 - Effect of callusing period on magnesium concentration of initial dry mass in different regions of Salt Creek cuttings.

Part	Concentration %			
	Initially	Callusing period (days)		
AR	a 0,208 A	a 0,207 A	a 0,208 A	a 0,226 A
SAR	a 0,227 A	a 0,210 A	a 0,207 A	a 0,228 A
SBR	a 0,227 A	a 0,214 A	a 0,203 A	a 0,226 A
BR	a 0,222 A	a 0,210 A	a 0,208 A	a 0,226 A

a : Compare in the same vertical column

A : Compare in the same horizontal row

Means accompanied by a common letter are not different at the 5% level

APPENDIX 31.2 - Effect of callusing period and the degree of callusing on magnesium concentration of initial dry mass in different regions of Salt Creek cuttings.

Callusing period (days)	Degree of callusing	Concentration (%)			
		AR	SAR	SBR	BR
14	Good	a 0,204	a 0,206	a 0,203	a 0,203
	Poor	a 0,213	a 0,207	a 0,203	a 0,213
21	Good	a 0,228	a 0,233	a 0,227	a 0,230
	Poor	a 0,224	a 0,222	a 0,225	a 0,222

a : Compare in the same vertical column seperately for each callusing period

Means accompanied by a common letter are not different at the 5% level

APPENDIX 32.1 - Effect of callusing period and degree of callusing on sugar, starch, hemicellulose, total N and soluble N as concentration of initial dry mass in Waltham Cross scions.

Component	Callus group	Percentage of initial dry mass: Initially	Callusing period 7	Callusing period 14	Callusing period 21
Sugar	Average	8,70 C	2,96 A	3,05 A	4,27 B
	Good callusing			a 3,02	a 4,39
	Poor callusing			a 3,08	a 4,15
Starch	Average	4,92 B	7,00 D	6,02 C	3,41 A
	Good callusing			a 5,91	a 3,12
	Poor callusing			a 6,13	b 3,72
Sugar + starch	Average	13,62 D	9,96 C	9,07 B	7,68 A
	Good callusing			a 8,93	a 7,51
	Poor callusing			a 9,21	a 7,87
Hemicellulose	Average	15,07 A	17,97 C	16,80 B	16,50 B
	Good callusing			a16,55	a16,28
	Poor callusing			a17,05	a16,73
Sugar + starch + hemicellulose	Average	28,69 D	27,93 C	25,87 B	24,18 A
	Good callusing			a25,48	a23,79
	Poor callusing			a26,26	a24,60
Total N	Average	0,606A	0,614A	0,608A	0,597A
	Good callusing			a 0,619	a 0,595
	Poor callusing			a 0,598	a 0,600
Soluble N	Average	0,108A	0,122A	0,148B	0,159B
	Good callusing			a 0,150	a 0,158
	Poor callusing			a 0,145	a 0,166

A - C : Compare in the same horizontal row

a, b : Compare in the same vertical column seperately for each component

Means accompanied by a common letter are not different at the 5% level

APPENDIX 32.2 - Effect of callusing period and degree of callusing on phosphorus, potassium, calcium and magnesium as concentration of initial dry mass in Waltham Cross scions.

Component	Callus group	Percentage of initial dry mass			
		Initially	Callusing period		
			7	14	21
Phosphorus	Average	0,112 B	0,107 B	0,106 B	0,100 A
	Good callusing			a 0,108	a 0,101
	Poor callusing			a 0,105	a 0,099
Potassium	Average	0,601 B	0,575 A	0,565 A	0,552 A
	Good callusing			b 0,586	a 0,565
	Poor callusing			a 0,545	a 0,538
Calcium	Average	0,592 A	0,560 A	0,567 A	0,549 A
	Good callusing			a 0,570	a 0,539
	Poor callusing			a 0,564	a 0,559
Magnesium	Average	0,170 A	0,165 A	0,163 A	0,155 A
	Good callusing			a 0,164	a 0,152
	Poor callusing			a 0,161	a 0,159

A - B : Compare in the same horizontal row

a, b : Compare in the same vertical column seperately for each component

Means accompanied by a common letter are not different at the 5% level

APPENDIX 33.1 - Seasonal changes in the dry mass per plant part and of the whole plant of Jacquez and Salt Creek during the nursery stage

Rootstock	Plant part	Dry mass (g) at each date													
		19 Aug	2 Sept	16 Sept	30 Sept	14 Oct	28 Oct	11 Nov	25 Nov	22 Dec	22 Jan	23 Feb	22 March	24 May	23 July
Jacquez	Leaves	Not determined seperately							1,03	2,22	6,25	5,90	6,34	1,01	0,00
	Shoots	Not determined seperately							0,23	0,44	1,92	2,50	2,92	3,14	3,30
	Leaves + shoots	0,00	0,00	0,01	0,13	0,19	0,40	0,62	1,26	2,66	8,17	8,40	9,26	4,15	3,30
	Stems	8,39	7,15	6,60	6,03	6,10	5,98	5,20	5,70	6,15	7,13	7,80	9,05	9,28	8,68
	Roots	0,00	0,00	0,00	0,00	0,02	0,07	0,29	0,63	2,21	7,03	8,55	12,60	9,82	10,69
	Whole plant	8,39	7,15	6,61	6,16	6,31	6,45	6,11	7,59	11,02	22,33	24,75	30,91	23,25	22,67
Salt Creek	Leaves	Not determined seperately							1,46	3,48	6,32	5,97	6,79	3,25	0,00
	Shoots	Not determined seperately							0,43	1,32	3,33	4,37	5,07	5,68	6,69
	Leaves + shoots	0,00	0,00	0,02	0,12	0,16	0,43	0,78	1,89	4,80	9,65	10,34	11,86	8,93	6,69
	Stems	7,30	6,73	7,10	6,77	6,24	6,05	5,90	6,20	6,80	7,42	8,89	9,10	9,17	9,42
	Roots	0,00	0,00	0,00	0,00	0,01	0,09	0,28	0,50	2,50	6,85	8,34	11,22	10,13	11,32
	Whole plant	7,30	6,73	7,12	6,89	6,41	6,57	6,96	8,59	14,10	23,92	27,57	32,18	23,23	27,43

APPENDIX 33.2 - Seasonal changes in the sugar, starch and hemicellulose as concentration of dry mass in the stem of Jacquez and Salt Creek plants during the nursery stage

Rootstock	Component	Percentage of dry mass at each date													
		19 Aug	2 Sept	16 Sept	30 Sept	14 Oct	28 Oct	11 Nov	25 Nov	22 Dec	22 Jan	23 Feb	22 March	24 May	23 July
Jacquez	Sugar	9,34	6,02	3,99	3,66	3,62	3,52	3,35	3,26	3,70	3,91	4,08	3,80	3,73	4,74
	Starch	6,26	6,72	7,08	6,03	4,04	2,08	1,29	1,40	3,85	5,80	9,51	10,22	10,86	9,43
	Sugar + starch	15,60	12,74	11,07	9,69	7,66	5,60	4,64	4,66	7,55	9,71	13,59	14,02	14,59	14,17
	Hemicellulose	22,95	23,46	21,49	23,34	23,52	23,11	24,90	22,49	24,45	23,72	23,24	22,25	21,58	18,36
	Sugar + starch + hemicellulose	38,55	36,20	32,56	33,03	31,18	28,71	29,54	27,15	32,00	33,43	36,83	36,27	36,17	32,53
Salt Creek	Sugar	7,40	5,09	4,11	3,74	2,78	2,97	2,39	2,39	3,43	3,66	3,53	3,35	3,68	4,10
	Starch	4,44	4,89	5,00	4,38	2,87	1,69	0,90	1,32	3,49	5,62	6,60	9,04	8,47	5,86
	Sugar + starch	11,84	9,98	9,11	8,12	5,65	4,66	3,29	3,71	6,92	9,28	10,13	12,39	12,15	9,96
	Hemicellulose	24,20	24,09	22,10	24,51	23,48	23,94	23,50	22,48	24,11	23,57	22,54	21,86	22,57	19,81
	Sugar + starch + hemicellulose	36,04	34,07	31,21	32,63	29,13	28,60	26,79	26,19	31,03	32,85	32,67	34,25	34,72	29,77

APPENDIX 33.3 - Seasonal changes in the sugar, starch and hemicellulose as concentration of dry mass in the shoots of Jacquez and Salt Creek plants during the nursery stage

Rootstock	Component	Percentage of dry mass at each date													
		19 Aug	2 Sept	16 Sept	30 Sept	14 Oct	28 Oct	11 Nov	25 Nov	22 Dec	22 Jan	23 Feb	22 March	24 May	23 July
Jacquez	Sugar	-	-	n a	n a	n a	n a	n a	5,67	3,87	5,55	4,72	4,09	4,66	8,54
	Starch	-	-	n a	n a	n a	n a	n a	5,20	4,46	4,46	7,66	8,07	7,89	4,46
	Sugar + starch	-	-	n a	n a	n a	n a	n a	10,87	8,33	10,01	12,38	12,16	12,55	13,00
	Hemicellulose	-	-	n a	n a	n a	n a	n a	12,04	13,99	17,04	20,08	19,95	20,00	17,14
	Sugar + starch + hemicellulose	-	-	n a	n a	n a	n a	n a	22,91	22,32	27,05	32,46	32,11	32,55	30,14
Salt Creek	Sugar	-	-	n a	n a	n a	n a	n a	3,88	3,44	5,00	3,94	3,61	3,83	6,25
	Starch	-	-	n a	n a	n a	n a	n a	4,90	3,92	3,34	5,51	4,96	5,96	3,05
	Sugar + starch	-	-	n a	n a	n a	n a	n a	8,78	7,36	8,34	9,45	8,57	9,79	9,30
	Hemicellulose	-	-	n a	n a	n a	n a	n a	9,86	16,66	17,42	19,40	19,89	19,08	17,97
	Sugar + starch + hemicellulose	-	-	n a	n a	n a	n a	n a	18,64	24,02	25,76	28,85	28,46	28,87	27,27

APPENDIX 33.4 - Seasonal changes in the sugar, starch and hemicellulose as concentration of dry mass in the roots of Jacquez and Salt Creek plants during the nursery stage

Rootstock	Component	Percentage of dry mass at each date													
		19 Aug	2 Sept	16 Sept	30 Sept	14 Oct	28 Oct	11 Nov	25 Nov	22 Dec	22 Jan	23 Feb	22 March	24 May	23 July
Jacquez	Sugar	-	-	-	-	n a	n a	n a	6,45	4,64	5,94	5,36	4,37	4,01	3,87
	Starch	-	-	-	-	n a	n a	n a	4,65	8,40	13,43	26,10	25,12	28,88	24,49
	Sugar + starch	-	-	-	-	n a	n a	n a	11,10	13,04	19,37	31,46	29,49	32,89	28,36
	Hemicellulose	-	-	-	-	n a	n a	n a	12,69	8,30	9,45	7,49	8,17	6,53	6,96
	Sugar + starch + hemicellulose	-	-	-	-	n a	n a	n a	23,79	21,34	28,82	38,95	37,66	39,42	35,32
Salt Creek	Sugar	-	-	-	-	n a	n a	n a	5,04	6,35	5,64	5,22	4,51	3,72	4,40
	Starch	-	-	-	-	n a	n a	n a	3,37	6,72	12,02	21,44	22,24	25,71	15,67
	Sugar + starch	-	-	-	-	n a	n a	n a	8,41	13,07	17,66	26,66	26,75	29,43	20,07
	Hemicellulose	-	-	-	-	n a	n a	n a	13,65	8,86	10,17	8,76	8,82	8,81	8,73
	Sugar + starch + hemicellulose	-	-	-	-	n a	n a	n a	22,06	21,93	27,83	35,42	35,57	38,24	28,80

n a : not analysed

APPENDIX 33.5 - Seasonal changes in the sugar, starch and hemicellulose content in the stems of Jacquez and Salt Creek plants during the nursery stage

Rootstock	Component	Amount per plant (g) on each date													
		19 Aug	2 Sept	16 Sept	30 Sept	14 Oct	28 Oct	11 Nov	25 Nov	22 Dec	22 Jan	23 Feb	22 March	24 May	23 July
Jacquez	Sugar	0, 783	0, 430	0, 265	0, 221	0, 221	0, 210	0, 174	0, 185	0, 227	0, 279	0, 318	0, 344	0, 346	0, 411
	Starch	0, 525	0, 480	0, 467	0, 364	0, 246	0, 124	0, 067	0, 080	0, 236	0, 413	0, 742	0, 924	1, 010	0, 818
	Sugar + starch	1, 308	0, 910	0, 732	0, 585	0, 467	0, 334	0, 241	0, 265	0, 463	0, 692	1, 060	1, 268	1, 356	1, 229
	Hemicellulose	1, 925	1, 677	1, 418	1, 407	1, 435	1, 382	1, 295	1, 282	1, 504	1, 691	1, 812	2, 036	2, 003	1, 594
	Sugar + starch + hemicellulose	3, 233	2, 587	2, 150	1, 992	1, 902	1, 716	1, 536	1, 547	1, 967	2, 383	2, 872	3, 304	3, 359	2, 823
Salt Creek	Sugar	0, 540	0, 342	0, 291	0, 253	0, 173	0, 179	0, 141	0, 148	0, 233	0, 271	0, 313	0, 304	0, 337	0, 386
	Starch	0, 324	0, 329	0, 355	0, 296	0, 179	0, 102	0, 054	0, 081	0, 237	0, 417	0, 586	0, 822	0, 776	0, 552
	Sugar + starch	0.864	0, 671	0, 646	0, 549	0, 352	0, 281	0, 195	0, 229	0, 470	0, 688	0, 899	1, 126	1, 113	0, 938
	Hemicellulose	1, 589	1, 621	1, 569	1, 659	1, 465	1, 448	1, 386	1, 393	1, 639	1, 748	1, 994	1, 989	2, 069	1, 866
	Sugar + starch + hemicellulose	2, 453	2, 292	2, 215	2, 208	1, 817	1, 729	1, 581	1, 622	2, 109	2, 436	2, 893	3, 115	3, 182	2, 804

APPENDIX 33.6 - Seasonal changes in the sugar, starch and hemicelluiose content in the shoots of Jacquez and Salt Creek plants during the nursery stage

Rootstock Cultivar	Component	Amount per plant (g) on each date													
		19 Aug	2 Sept	16 Sept	30 Sept	14 Oct	28 Oct	11 Nov	25 Nov	22 Dec	22 Jan	23 Feb	22 March	24 May	23 July
Jacquez	Sugar	-	-	n a	n a	n a	n a	n a	0,013	0,017	0,106	0,118	0,119	0,146	0,281
	Starch	-	-	n a	n a	n a	n a	n a	0,012	0,019	0,085	0,191	0,235	0,247	0,147
	Sugar + starch	-	-	n a	n a	n a	n a	n a	0,025	0,036	0,191	0,309	0,354	0,393	0,428
	Hemicellulose	-	-	n a	n a	n a	n a	n a	0,027	0,061	0,327	0,502	0,582	0,628	0,565
	Sugar + starch + hemicellulose	-	-	n a	n a	n a	n a	n a	0,052	0,097	0,518	0,811	0,936	1,021	0,993
Salt Creek	Sugar	-	-	n a	n a	n a	n a	n a	0,016	0,045	0,166	0,172	0,183	0,217	0,418
	Starch	-	-	n a	n a	n a	n a	n a	0,021	0,051	0,111	0,240	0,251	0,338	0,204
	Sugar + starch	-	-	n a	n a	n a	n a	n a	0,037	0,096	0,277	0,412	0,434	0,555	0,622
	Hemicellulose	-	-	n a	n a	n a	n a	n a	0,042	0,219	0,580	0,847	1,008	1,083	1,202
	Sugar + starch + hemicellulose	-	-	n a	n a	n a	n a	n a	0,079	0,315	0,857	1,259	1,442	1,638	1,824

n a : not analysed

APPENDIX 33.7 - Seasonal changes in the sugar, starch and hemicellulose content in the roots of Jacquez and Salt Creek plants during the nursery stage

Rootstock	Component	Amount per plant (g) on each date													
		19 Aug	2 Sept	16 Sept	30 Sept	14 Oct	28 Oct	11 Nov	25 Nov	22 Dec	22 Jan	23 Feb	22 March	24 May	23 July
Jacquez	Sugar	-	-	-	-	n a	n a	n a	0,040	0,120	0,417	0,458	0,550	0,393	0,413
	Starch	-	-	-	-	n a	n a	n a	0,029	0,185	0,944	2,231	3,165	2,836	2,618
	Sugar + starch	-	-	-	-	n a	n a	n a	0,069	0,287	1,361	2,689	3,715	3,229	3,031
	Hemicellulose	-	-	-	-	n a	n a	n a	0,079	0,183	0,664	0,640	1,029	0,641	0,744
	Sugar + starch + hemicellulose	-	-	-	-	n a	n a	n a	0,148	0,470	2,025	3,329	4,744	3,870	3,775
Salt Creek	Sugar	-	-	-	-	n a	n a	n a	0,025	0,158	0,386	0,435	0,506	0,376	0,498
	Starch	-	-	-	-	n a	n a	n a	0,016	0,168	0,823	1,788	2,495	2,604	1,773
	Sugar + starch	-	-	-	-	n a	n a	n a	0,041	0,326	1,209	2,223	3,001	2,980	2,271
	Hemicellulose	-	-	-	-	n a	n a	n a	0,068	0,221	0,696	0,730	0,989	0,892	0,988
	Sugar + starch + hemicellulose	-	-	-	-	n a	n a	n a	0,109	0,547	1,905	2,953	3,990	3,872	3,259

n a : not analysed

APPENDIX 34.1 - Effect of growth period and IBA treatment on the whole plant dry mass of Jacquez and Salt Creek

Rootstock and treatment	Amount per plant (g)						Percentage change from initial value after 56 days
	Growth period (days)						
	0	7	14	28	42	56	
Jacquez control	a 5,93	a 5,80	a 5,72	a 5,73	a 5,45	a 5,45	- 10,6
Jacquez IBA	a 5,93	a 6,03	a 5,74	a 5,60	a 5,49	a 5,36	- 9,6
Salt Creek control	a 5,92	a 5,99	a 5,76	a 5,70	a 5.61	a 5,43	- 8,3
Salt Creek IBA	a 5,92	a 5,84	a 5,77	a 5,62	a 5.55	a 5,47	- 7,6

APPENDIX 34.2 - Effect of growth period and IBA treatment on the stem dry mass of Jacquez and Salt Creek plants

Rootstock and treatment	Amount per plant (g)						Percentage change from initial value after 56 days
	Growth period (days)						
	0	7	14	28	42	56	
Jacquez control	a 5,93	a 5,80	a 5,68	a 5,58	a 5,25	a 5,09	-14,2
Jacquez IBA	a 5,93	a 6,03	a 5,72	a 5,46	a 5,28	a 5,08	-14,3
Salt Creek control	a 5,92	a 5,99	a 5,73	a 5,58	a 5,42	a 5,20	-12,2
Salt Creek IBA	a 5,92	a 5,84	a 5,75	a 5,52	a 5,35	a 5,15	-13,0

a : Compare in the same vertical column seperately for each cultivar. Means accompanied by a common letter are not different at the 5% level.

APPENDIX 34.3 - Effect of growth period and IBA treatment on the dry mass of shoots and leaves of Jacquez and Salt Creek plants

Rootstock and treatment	Amount per plant (mg)			
	Growth period (days)			
	14	28	42	56
Jacquez control	a 34	a 140	a 193	a 200
Jacquez IBA	a 19	a 135	a 212	a 262
Salt Creek control	a 27	a 107	a 179	a 226
Salt Creek IBA	a 15	a 100	a 174	a 253

APPENDIX 34.4 - Effect of growth period and IBA treatment on the dry mass of roots of Jacquez and Salt Creek plants

Rootstock and treatment	Amount per plant (mg)		
	Growth period (days)		
	28	42	56
Jacquez control	-	0,7	3,1
Jacquez IBA	-	3,1	20,4
Salt Creek control	-	3,3	11,3
Salt Creek IBA	0,4	27,6	54,7

a : Compare in the same vertical column separately for each cultivar

Means accompanied by a common letter are not different at the 5% level.

METHODS FOR EXTRACTION OF SUGAR, STARCH AND HEMICELLULOSE IN GRAPEVINE SAMPLES

Sugar

Weigh ca 0, 5 g of the sample accurately, transfer to a 100 ml Erlenmeyer flask, add 50 ml 80% ethanol (room temperature), stopper and shake for 15 hours. Filter off the extract and wash the residue 3 times with small portions of 80% ethanol. Evaporate the filtrate on a stem bath in a shallow 250 ml beaker until no alcohol can be smelt. Before all the alcohol is evaporated, wash the sides of the beaker with about 10 deionised water. This helps to clean the sides and prevents the solution becoming too concentrated. Wash the concentrate into a 100 ml volumetric flask, add 10 ml Ba $(OH)_2$ solution, shake and while shaking, add the Zn SO_2 solution as determined by titration. Fill to the mark with deionised water, shake and filter. Transfer 20 ml filtrate to a 100 ml wide mouth Erlenmeyer, add 2, 5 ml 66% HCl (= 1N HCl in solution), cover with a watch glass and boil in a covered steam bath for 30 minutes. Cool down, neutralise with 2 ml 6N NaOH and finally with 1N NaOH to a pH of 7-7, 5. Add 2 ml glycerol-C, shake, filter into a 100 ml volumetric flask, wash the residue twice with deionised water, fill up to the mark with deionised water and take a sample. The sample should be colourless. It can be stored under re= frigeration for a maximum of 5 days. For longer periods a crystal of thymol must be added.

Starch

Wash the residue of the sugar extract in the filter into a 100 ml Erlenmeyer using 20 ml deionised water, and put onto a steam bath for 30 minutes. After cooling add 20 ml buffer solution and 20 ml taka-diastase enzyme in this order. Stopper, shake lightly, and put into an oven at $37^{o}C$ for 44 hours. Prepare two blanks with water, buffer and enzyme, put into the oven and treat the same as the samples from thereon.

After removal from the oven, filter into a 250 ml volumetric flask, add 20 ml $Ba(OH)_2$ solution, shake and while shaking add $Zn SO_4$ solution as determined by titration. Fill the flask to the mark, shake and filter. Transfer 20 ml filtrate to a 100 ml wide mouth Erlenmeyer, add 0,8 ml 5N H_2SO_4 (= 0,2 N $H_2 SO_4$ in solution) and boil in a covered steam bath for 30 minutes. Cool down, neutralise with 6N Na OH and finally 1N NaOH to pH 7-7,5. Add 2 ml glycerol - C, shake, filter into a 100 ml volumetric flask, fill to the mark with deionised water and take a sample. Store as for sugar.

Hemicellulose

Wash the residue of the starch extract in the filter into a 500 ml boiling flask using 20 ml deionised water, add another 160 ml water and 20 ml 66% HCl. Add ca 0,3 g activated C (deactivation is obtained by boiling in HCl) and boil under reflux for 3 hours. Cool down, filter and cool down to room temperature. Neutralise to pH 7-7,5 with 17 ml 6N NaOH and finally 1N NaOH. Wash into a 1 l volumetric flask, add 20 ml $Ba(OH)_2$ solution, shake and while shaking add the $Zn SO_4$ solution as determined by titration. Fill the flask to the mark with water, shake, filter, take a sample and dilute with an equal volume of deionised water. Store as for sugar.

Dilution factors

For the dilutions described the following dilution factors must be used:

Sugar	0,1
Starch	0,225
Hemicellulose	0,4

The starch factor includes a factor of 0,9 for conversion of the glucose concentration to starch concentration.

MATERIALS FOR EXTRACTION OF SUGAR, STARCH AND HEMICELLULOSE AND DETERMINATION OF REDUCING SUGARS

1. Buffer solution pH 4.45

1.1 0, 2 N acetic acid: 12 ml glacial acetic acid made up to 1 l deionised water.

1.2 0, 2 N sodium acetate: 27, 3 anhydrous sodium acetate made up to 1 l with deionised water.

Mix 3 vols of 1.1 with 2 vols of 1.2. Add 1 g thymol/1 of buffer.

2. Taka-diastase enzyme

20 g "Chlarase 1500" (Miles Serevac) is dissolved in 750 ml deionised water and dialysed for 28 hours in a no 36 (45 mm width) dialysis membrane (Union Carbide) in running tap water. It is then filtered and made up to 1 l with deionised water.

3. Reagents for digesting and neutralising

3.1 66% HCl: 660 ml conc. HCl made up to 1 l with deionised water.

3.2 5 N $H_2 SO_4$: 139, 1 ml conc. $H_2 SO_4$ made up to 1 l with deionised water.

3.3 6 N NaOH: 240 g anhydrous NaOH made up to 1 l with deionised water.

3.4 1 N NaOH: 40 g anhydrous NaOH made up to 1 l with deionised water.

4. Clearing reagents

4.1 Ba $(OH)_2$ solution: 56, 8 g Ba $(OH)_2 . 8 H_2O$ made up to 1 l with deionised water.

4.2 Zn SO_4 solution: 53, 6 g Zn $SO_4 . 7 H_2O$ made up to 1 l with deionised water.

Add the Ba $(OH)_2$ solution to a 40 ml aliquot of the Zn SO_4 solution while stir= ring vigorously until a pH of 8, 3 is obtained. Repeat twice and determine the average ratio: These solutions must be used in the ratio obtained in this way.

5. Decolouring reagent

Mix 135 g Darco G60 activated C with 900 ml 50% watery glycerol. 2 ml of this mixture is equivalent to 0,3 g C.

6. Reagents for determination of reducing sugars

6.1 Alkaline ferricyanide solution: 1,3 g $K_3(Fe(CN)_6)$ plus 20 g anhydrous Na_2CO_3 made up to 1 l with deionised water. Add 1 ml Brij-35.

6.2 KCN solution: 5 g KCN made up to 1 l with deionised water.

7. Stock and standard solutions

7.1 Glucose stock solution (1 000 mg/l): 1,1 g glucose monohydrate made up to 1 l with deionised water.

7.2 Glucose standard solutions: A range of 10 to 100 mg/1 standard solutions is prepared by making up 1 - 10 ml aliquots from the stock solution to 100 ml.

8. Filter paper

Whatman No 2.